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LABORATORY MEDICINE

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Guest Editorial *Laboratory Medicine-Past, Present And Future*

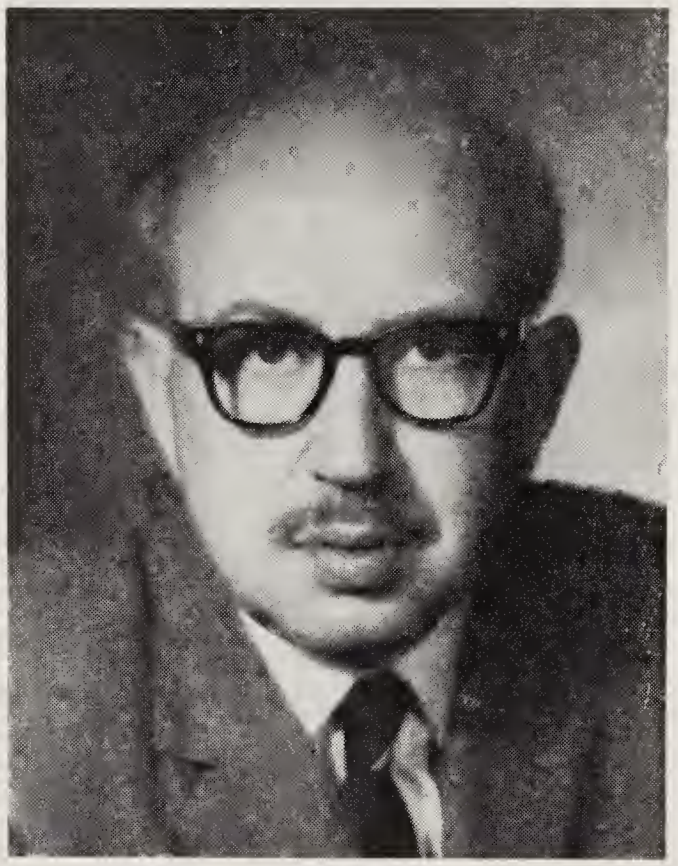
NICHOLAS FORBATH
M.D., M.A., F.R.C.P.(C). Born on May 21, 1929 in Budapest, Hungary.

Dr. Forbath attended the University of Budapest from 1947 until 1953, where he obtained his degree in medicine. From 1954 until 1956 he was a lecturer in Physiology at the University of Szeged in Hungary. In December 1956 he left Hungary under some duress and on Dr. Charles H. Best's invitation came to Toronto in 1957. He worked at the Banting and Best Department of Medical Research until July 1958 and received his M.A. degree in Physiology.

His internship at the Toronto General Hospital 1958 - 1959 was followed by residency training in medicine in Toronto. In 1962 he became F.R.C.P.(C) and the following year he joined the staff of the Department of Medicine in the Toronto General Hospital while maintaining his connections with the Department of Physiology.

From 1964 to 1969 he was a Research Scholar of the Medical Research Council. His main scientific interest is in diabetes and carbohydrate metabolism. At present he is Assistant Professor in the Department of Medicine, and he is active both in the clinical and in the research field.

Dr. Forbath is married to a physician and has a son and a daughter.

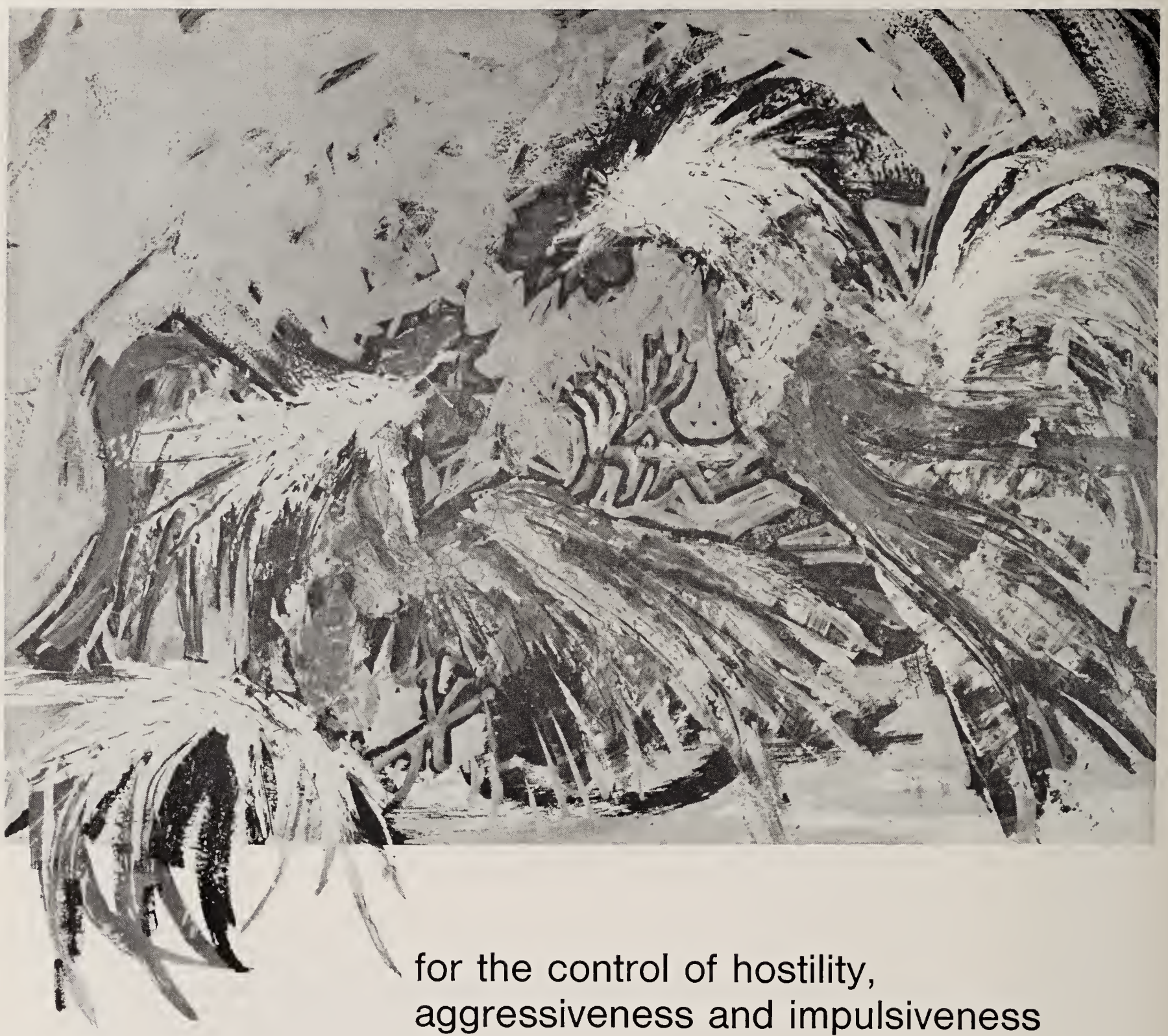


While medicine and surgery can look back over many thousands of years of history, laboratory medicine and especially clinical chemistry is a relatively new branch of the healing art. None of the tests that are routine today were available a century ago. Claude Bernard in the middle of the 19th century used several ounces of blood for a single blood sugar determination. Plasma Na and K estimations were regarded as special procedures 25 years ago and were available only in major centres. Indeed few surgeons have contributed more to the management of electrolyte disorders and to the care of post-operative patients than the inventor of the flame photometer which made the determination of plasma Na and K quick, accurate and inexpensive. The tremendous progress in physics and chemistry in the last 100 years laid the foundation of physiology and biochemistry and these in turn prepared the ground for modern day laboratory technology.

We teach that thoroughness and meticulous attention to detail in history taking and physical examination is the foundation of good medicine. The intelligent interpretation of the data so accumulated forms the basis of diagnosis and decision making. Laboratory investigation is in essence the extension of the physical examination. The microscope and the autoanalyzer make visible, alterations in the body which are otherwise inaccessible to our special senses. Thus the extension of the principle of thoroughness and attention to detail in laboratory investigation would mean that we must perform and repeat each and every test on every patient. This notion seems absurd to the older generation of doctors but it is perfectly logical. Inspite of its logic the impracticability of this approach is obvious. It's end results are haemorrhagic shock and bankruptcy.

In 1969 one of the main concerns of the medical profession is the most efficient utilization of the laboratory. The cost of laboratory investigation is skyrocketing. More and more tests are ordered per patient each year and the laboratory demands a constantly increasing share of the total health care dollar. Since the latter is a finite quantity, by spending more on laboratory investigation we inevitably have less to spend on other services. The curriculum and the textbooks do not deal with the indications for laboratory investigation as they deal with the indications for surgical procedures. This is understandable because to lay down specific rules in such a rapidly developing discipline is impossible. The question the doctor has to ask himself when he orders a test is "How may this result influence the management or the prognosis of this patient?" The test should be ordered if there is a reasonable chance that the answer to this question is in the affirmative.

To prophesy on the future of laboratory medicine borders on science fiction. The real progress will not be merely more blood tests on smaller samples at a lesser cost but more meaningful tests. Still most of the results of our investigations are at best indirect evidence of the disease. The alterations we detect are too remotely related to the basic pathology. Our investigative methods are getting closer to the basic disease process with the advancement of science. The progress from the measurement of basal metabolic rate through the determination of PBI to the estimation of serum thyroxine and free thyroxine in the diagnosis of thyroid dysfunction illustrates this process. It is hoped that a similar advance will be made in the diagnosis of other diseases. And this is a very promising future.



for the control of hostility,
aggressiveness and impulsiveness

a new psychotropic agent

NEULEPTIL

pericyazine

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℞ NEULEPTIL
pericyazine

Neuleptil is a phenothiazine of the piperidine group with sedative properties and a mild antipsychotic activity.

Indications: as an adjunct in some psychotic patients, for the control of hostility, impulsiveness and aggressiveness.

Dosage: ADULTS - 5 to 20 mg at mid-day and 10 to 40 mg in the evening; when prolonged treatment is required: lower doses, i.e.: 5 to 15 mg at mid-day and 5 to 30 mg in the evening; generally, the lower dosage is used initially and increased to the effective level. In the elderly patients, average dose of 5 mg per day, progressively increased until response is obtained.

Contraindications: circulatory collapse, comatose states, deep sedation, particularly when they are caused by CNS depressants; history of blood dyscrasias, liver disease or hypersensitivity to phenothiazines; should not be associated with spinal or regional anesthetics.

Adverse reactions: drowsiness, reactions of the ANS, postural hypotension and psychomotor troubles may appear at the beginning of treatment but they are usually transient or subside upon adjustment of dosage; extrapyramidal reactions may occur mainly with high doses. One should bear in mind the possibility of the side effects due to phenothiazines: blurred vision, dryness of the mouth, constipation, nasal congestion, postural hypotension, cardiac arrhythmias, parkinsonism, dystonias, blood dyscrasias, agranulocytosis, skin reactions, photosensitivity, abnormal pigmentation, lactation, weight gain, edema.

Precautions: caution should be exercised in elderly or debilitated patients, in cases of glaucoma or prostatic hypertrophy and when the drug is associated with CNS depressants, the doses of which should be reduced by half. Patients should be instructed not to drive a car or operate machinery if and as long as they feel drowsy. The safety of Neuleptil in pregnant women and in children has not yet been established.

Treatment of overdosage: symptomatic (no specific antidote); gastric lavage or administration of an emetic; if circulatory collapse occurs, routine treatment of shock and noradrenaline, if necessary.

Dosage forms: capsules of 5 and 10 mg.

Poulenc

The Hospital Laboratory

David Clarkson 7T0

INTRODUCTION

Medical practitioners are making increasing use of diagnostic laboratory facilities, which have become a major item in the time and cost involved in providing health services. This article offers a description of the organization of the "hospital laboratory" which we use, often with so little thought. In addition, there will be some speculation of the change the diagnostic laboratory is likely to undergo.

ORGANIZATION

At the head of the laboratory and theoretically responsible for the standard of its work is the Director of Laboratories. The director is a doctor – often a pathologist, but in larger hospitals hematologists and other specialists share in laboratory administration. The director is a member of the Heads of Department Committee, the medical staff and often the Medical Advisory Board of the hospital.

The laboratory is subdivided, usually into five departments: Blood Bank, Chemistry, Hematology, Bacteriology, and Pathology (surgical and morbid). Depending on the size of the laboratory there may be a chief technologist over each individual department, or one chief technologist over five departments. This chief technologist is responsible to the director. However, the actual laboratory determinations are performed by technicians.

The Registered Technologist in Ontario is one who has taken two years of training after grade thirteen or equivalent. The first year is basically didactic teaching at the Institute of Technologists in Toronto. The second year is a sort of rotating internship through the various departments where the student has previously been accepted. In the inevitable examinations which follow this second year, approximately eighty-five per cent of the candidates pass on their first attempt and subsequently become registered.

Further formal education is possible for the Registered Technologist. After working for three years, the R.T. may spend a year studying in one specialty or more, and undergo examinations for the degree of Advanced Registered Technologist (A.R.T.). About forty per cent of candidates seeking this degree are awarded it on their first attempt. A further degree, called the Micentia, is possible. It is awarded after additional study and practical experience. It may be in a specialty or in the field of laboratory administration. About one hundred Canadians hold this degree.

This is not to imply that everyone performing laboratory determinations has some sort of formal education

beyond high school. Indeed many technicians working in hospital laboratories in downtown Toronto hospitals hold no formal degree. They have however worked in a particular area – often for many years – and have presumably become proficient at a given number of tests. This does not apply to the same degree in many of the newer suburban hospitals where a much higher ratio of "degrees" to "non-degrees" exists.

COSTS

When considering the cost of operating a hospital laboratory there are many factors to consider. Obviously there are administrative mechanisms to maintain, technologists to pay and reagents to buy. One however must also include the cost of sophisticated auto-analyzers (and their constant repair bills), the cost of occupying valuable land (especially in downtown Toronto), building depreciation and the interest lost on the large capital investments.

Although most of these factors are constantly rising, the cost of laboratory tests is falling, due to improved methods and automation. Consider some representative costs at the Toronto General Hospital:

Automated	
Blood Sugar	$ .73
S G O T	$ .63
Alkaline Phosphatase	$ 1.54
Electrolytes	$ 1.80
Non-automated	
Serum Renin	$100.00
Aldosterone	$ 60.00
T.S.H.	$ 85.00
Plasma Cortisol	$ 28.00

Although automated, tests only deceptively appear fairly inexpensive to perform — the Toronto General Hospital spent $115,000.00 in 1968 on electrolyte determinations alone. In addition the volume of work is constantly increasing. One prominent laboratory director says that if the demand on laboratory facilities continues to accelerate at the present rate even the new 24 channel auto-analyser (cost: one half million dollars each) will not be able to keep pace.

This observation leads to a brief consideration of possible radical changes in the hospital laboratory as it is today, *viz* The In-Common Laboratory.

IN COMMON LABORATORY

The I.C.L. is an experimental attempt, financed by the Ontario Health Services Commission to find ways of combining the work of many hospitals in order to make testing more economical and more accurate.

In its present form at the New Mount Sinai Hospital it is subscribed to by about six Toronto area hospitals. These hospitals collect their samples and send them to the I.C.L. The I.C.L. is fully automated and ideally the machines are always standardized and running; hence the sample can be quickly dealt with and the result returned by telephone if speed is important. Telex is also being considered.

Determinations being done at present are:

i serum uric acid
ii serum creatinine
iii organic phosphate
iv cholesterol
v P.B.I.
vi serum iron and iron binding capacity

This project may prove superior to existing set-ups with respect to cost and accuracy.

Ultimately, O.H.S.C. pays the bill for diagnostic laboratory services. It is therefore the total laboratory work which is important, not the cost to the hospitals taken individually. It seems quite reasonable then that money could be saved on a test such as serum uric acid which can be automated and which is done relatively infrequently by doing several hospitals' requirements on one machine, by one technologist. In this sense the I.C.L. is very much an experiment, still at the stage of finding out what procedures can be most economically combined.

It is clear that the statistical significance in the interpretation of one's results increases as the number of samples in the test increases. At the I.C.L., it is hoped that enough samples can be processed daily to allow calculation of meaningful limits of normal variation. This is often not possible in an individual hospital if only three or four tests are done daily.

It is also possible for the I.C.L. to employ computers to analyze results in situations an individual hospital would not. In fact the system used at the I.C.L. at present has a built-in correction for "operator bias". Even though the tests are automated, it is still possible for the operator to unknowingly influence the results. If an operator's results are continuously followed, her "handicap" can be ascertained, and a correction factor is automatically applied by giving the computer the operator's identity along with the raw data.

FUTURE

At present, the I.C.L. is experimental, although its organizers are enthusiastic about its initial success. It is a nine to five, Monday to Friday proposition. But its organizers hope that it may be able to operate on a seven day week, twenty-four hour day basis – equipped to handle routine and emergency work. With rapid transit and modern communications this is realistic. Certainly, the present system of having to call a technician at night for an emergency test in many hospitals is far from satisfactory. Time is required for the technician to come to the hospital, set up and standardize the equipment, run the test, and calculate the results. Often a satisfactory control is not obtained the first time. It is easy to share in the I.C.L. enthusiasm with optimism.

Glandular Function Tests

J. W. F. Cowell, M.Sc., 7T0

The abnormally functioning endocrine gland manifests itself in a characteristic set of signs, symptoms and laboratory values.

How does one assess the function of a gland by the use of the laboratory? There are several approaches by which one can measure the glandular product either directly or indirectly.

The following article is a short resumé and critical assessment of the more useful tests available in the hospital setting in assessing the function of:

I. The Thyroid Gland
II. The Adrenal Cortex
III. The Adrenal Medulla
IV. The Pituitary Gland
V. Other Glands

A. The Pancreas
B. The Gonads; The Fetal – Placental Unit
C. The Parathyroid Gland

I THE THYROID GLAND

(a) Normal Hormone Leves
i. Serum Thyroxine (T_4) 0.3ug%
ii. Serum Triiodothyronine (T_3) 0.3ug%

(b) Tests of Thyroid Function (1) (2)
i. Protein Bound Iodine (PBI)

This test is essentially a measure of the circulating thyroxine bound to thyroxine binding globulin (TBG).

Normal range 4-8ug%
Hyperthyroidism 7-10ug%
Hypothyroidism 3-5ug%

The usefulness of this test is diminishing as better methods become available for measuring T_4 directly.

The PBI is beset by many problems. The patient must not have been exposed to any iodine containing compounds before the test. Aqueous pyelographic drugs such as Diodrast or Hippuran will disturb the test for up to 2 weeks; choleocystographic dyes such as Telepaque for 3-6 months; Lipoidal or Pantopaque for myelography or brochography for 1-5 years. The PBI level is affected by the level of TBG. For example, in pregnancy the PBI level will be falsely high due to the high level of TBG. Conversely the level of the PBI will be falsely low in a state of malnutrition.

ii. I^{131} uptake

This test characterizes the iodine trapping mechanism of the thyroid.

Technique: An oral tracer dose of Na I^{131} (5-15uc) is given. Twenty-four hours later the radioactive uptake by the thyroid is measured.

Normal range .....10-40% of dose
Hyperthyroidism .....45% of dose
Hypothyroidism .....10% of dose

This test as well is beset by problems. For example, if the turnover of iodide in the thyroid is very rapid the test may reflect a normal uptake. In addition iodide exposure and TBG levels will interfere with the accuracy of the test. Here again Diodrast and Hippuran will disturb the test for up to 4 weeks, Telepaque for up to 4-6 months, Lipoidal and Pantopaque for up to 1-5 years.

iii. Serum T_3 Uptake Test

This test measures the amount of TBG saturation, thereby reflecting the effective amount of T_4 bound to the TBG.

Principle: TBG has many more receptor sites on it than are required to carry T_4 under normal conditions. Even in the hyperthyroid state there are some unoccupied receptor sites. Using an anionic resin which will also bind T_3, one can determine the relative saturation of TBG. If there is a high level of T_4 there will be only a few receptor sites available to T_3 for binding, thus more will be taken up by the resin. The reverse is true for the hypothyroid state. Since this test depends upon a normal level of TBG, states in which the level of TBG is abnormal will invalidate the results.

Technique: One ml. of patient serum is allowed to interact with a known amount of I^{131} labelled T_3. The amount of I^{131} bound to the anionic resin after the reaction with the TBG in the patient's serum sample is taken as a percentage of the total amount of I^{131} T_3 which could have taken up the resin.

Thus in the Hyperthyroid state the percentage is high because little I^{131} T_3 would be taken up by the already well-saturated patient's TBG.

Normal .....30 ± 5%
Hyperthyroidism .....45 ± 10%
Hypothyroidism .....25 ± 3%

iv. Serum T_4

There is available a method[3] to measure directly the total serum T_4. There is also a test for measuring the minute amount of *free* T_4, but it is not yet practical for the routine laboratory.

Principle: The principle for measuring total serum T_4 is much the same as for the Serum T_3 uptake test and by using a series of T_4 standards the actual amount of T_4 can be calculated.

Normal .....4-11ug% Mean 6.2ug%
Hyperthyroid .....mean 13.5ug%
Typothyroid .....mean 2.0ug%

In pregnancy there will be slightly elevated levels and in malnutrition or protein depletion there will be a decreased level.

v. Special Tests

The TSH test will differentiate between primary myxedema and that due to Anterior Pituitary malfunction. A baseline I^{131} uptake is determined. Then 5-10 units of TSH are given intramuscularly for three consecutive days, and once again the I^{131} uptake is repeated. In the normal individual there is a significant increase in uptake, while in primary hypothyroidism the uptake remains low. If the condition is secondary to Anterior Pituitary dysfunction there is a significant rise in uptake. With this test there is some risk of cardiovascular adverse effect and possible adrenal insufficiency.

vi. T_3 Suppression Test

This test differentiate between thyrotoxicosis due to Long Acting Thyroid Stimulator (LATS) and other forms of Hyperthyroidism.

Technique: 25ug of T_3 is given every eight hours for 2 days. This regimen suppresses the I^{131} uptake normally, however there is no suppression if the thyrotoxicosis is due to LATS.

vii. Thyroid Scan

Here relatively higher doses of Na I^{131} are given and the thyroid is scanned by a gamma counter. From this a map of the thyroid can be drawn. In areas of no uptake (cold nodules) there is non-functioning tissue. In areas of increased uptake (hot-nodules) there is hyperfunctioning tissue. When this is combined with the T_3 suppression test, autonomous nodules, i.e. not depending on TSH stimulation can be shown to exist.

II THE ADRENAL CORTEX[4]

(a) Direct methods of measuring hormone secretion

There are complex isotopic techniques for measuring the daily secretion rates of hydrocortisone, corticosterone, and aldosterone, but they are not widely available. These measurements are useful however for determining the normal diurnal vaiation, i.e.

8 a.m. 4-10ug%
5 p.m. 2-5ug%

(b) Indirect Methods

These methods involve principally the measurement of hormone metabolites in 24 hour urine collections.

i. Urine 17-OH steroids: This test measures about 25% of the metabolites of hydrocortisone. Normal range: 2-10mg/day.

ii. Urine 17-Keto Steroids: In the female all of the urinary 17-KS come from the adrenocortical androgens. In the male 2/3 come from the adrenal cortex and 1/3 from the testes.

Normal Adult Range
Female ..... 6-16mg/24hrs.
Male ..... 10-22mg/24hrs.

iii. Aldosterone: Here 18-glucuronide, a unique metabolite of aldosterone, is measured. The measurement accounts for about 10% of the secreted aldosterone. Normal range ..... 5-20ug/day.

(b) Function Tests

i) ACTH Test

This test is useful in the diagnosis of primary adrenocortical insufficiency. In this condition there is no rise in urinary steroids in response to ACTH.

Technique: The whole test lasts 4 days with 24-hour urine samples collected for 2 days prior to ACTH administration and for 2 days during administration. ACTH is administered as 40 units in 500 ml. 5% dextrose in water intravenously over an eight hour period from 9 a.m. to 5 p.m.

Principle: The principle of this test is exactly the same as that of the TSH test, that is, to test whether hypofunctioning of the gland is due to lack of stimulation by ACTH from the pituitary or a fault in the gland itself.

If there is a significant increase in 17-OH steroids in the urine after the administration of ACTH, then the fault lies in the pituitary not in the adrenal cortex.

ii. Metyrapone (Metopirone) Test

Use: This test is used in the diagnosis of secondary adrenocortical insufficiency, and as such is really a test of pituitary function.

Technique: For four consecutive days, twenty-hour urine collections are made. On the third day 750 mg metyrapone is given orally every four hours.

Principle: Metyrapone selectively blocks 11 - Beta hydroxylation and thereby prevents the formation of hydrocortisone. The inhibition of hydrocortisone on the pituitary having been removed there is increased ACTH production and an increased amount of hydrocortisone precursors appearing in the urine, measured as 17-OH steroids. This test can also be used to differentiate adrenal hyperplasia and adenocarcinoma of the adrenal cortex. In patients with a tumour there will be no change in urinary 17-OH steroids since the self-sufficient, hydrocortisone procuring tumour has long since maximally reduced pituitary ACTH.

iii. Dexamethasone Suppression Test

Dexamethasone is a potent inhibitor of hypothalamic release of Corticotropin Releasing Factor (CRF).

i. Small Dose Test

Use and Principle: This test although not particularly reliable is sometimes used to distinguish between patients with Cushing's syndrome and patients with simple obesity. Patients with Cushing's syndrome always have elevated urinary 17-OH steroids, while patients with simple obesity occasionally do. Dexamethasone in small doses suppresses urinary 17-OH steroids to below 4 mg./day in obesity, while in Cushing's syndrome there is no suppression.

Technique: Four consecutive 24 hr. urine collections are made and 17-OH steroids measured. On days 3 and 4 Dexamethasone is given. The dose is 0.5mg orally every four hours.

ii. Large Dose Test

Use: This test which is more reliable than the small dose test is used to distinguish beteen adrenal tumour and adrenal hyperplasia whether they be hydrocortisone- or androgen-secreting. Since an adenocarcinoma of the adrenal cortex is not under the control of ACTH, there will be no fall in urine 17-OH steroids as there would be with adrenal hyperplasia which is under the control of ACTH.

Technique: The technique is the same as for the small dose test except that 20 mg. of Dexamethasone is given. If an androgen secreting tumour is suspected, 17KS are measured in the urine.

iv. Plasma Renin Activity[5]

This parameter is useful in determining the difference between the syndrome of increased aldosterone production and hypokalemia associated with hypertension caused by primary aldosteronism (Conn's syndrome) and the same syndrome caused by functional renal hemodynamic change. Suppression of Renin activity in combination with supernormal production of aldosterone is characteristic of primary aldosteronism. Patients with Conn's syndrome are very sensitive to infusion of angiotensin as well.

III ADRENAL MEDULLA

The important test here, is used to diagnose the presence of a pheochromocytoma (90% of which occur in the adrenal Medulla). This tumour causes a rare but curable form of hypertension. The best test is to measure the Vanillylmandelic Acid (VMA) level in the urine.

Normal ..... 2-7 mg./24hrs.

N.B. Coffee, fruit and vanilla in the diet may interfere with the test.

IV THE PITUITARY GLAND

A. Anterior Pituitary

i. TSH: This was discussed with the thyroid gland. There is being developed a Radioimmunoassay (RIA) for this substance.

ii. ACTH: This was discussed with the Adrenal Cortex. No test is available to quantitate this substance.

iii. Growth Hormone (GH): This hormone is measured using RIA technique.

(RIA). Normal ..... children - 4-8ng/ml.
adults - 2-6ng/ml.
Acromegaly ..... 5-12ng/ml.

iv. FSH and LH: There is a RIA being developed for these hormones in the blood. Also a urine FSH bioassay is available for differentiating between hypogonadotrophic hypogonadism and secondary hypogonadism.

Normal ..... 7-50MU/24 hr. urine
Primary hypogonadism ..... 780MU/24 hr. urine
Secondary to Anterior Pituitary .... 6 MU/24 hr. urine

v. Prolactin: There is a RIA available but it is not a practical test.

vi. MSH: There is a RIA available but it is not a practical test.

vii. Panhypopituitarism (PHP)

(1) GH. is a useful hormone to measure here since it is one of the last hormones to disappear in a failing pituitary.

(2) The ability to respond to hypoglycemia is markedly decreased in PHP. 0.05-0.1 u/kg. of insulin is given IV and a series of plasma glucose levels are recorded. There is a marked increase in response to insulin, as reflected by the marked hypoglycemia.

(3) Pitressin Test: Here again the response is markedly depressed as compared to normal.

* MU = mouse unit.

B. *Posterior Pituitary*

The most important test here is the IV Saline test to differentiate Diabetes Insipidus and Psychogenic polydypsia and polyuria. 2.5% saline is infused in an arm vein for 45 minutes. If there is a significantly decreased urine volume then the condition is psychogenic.

V. OTHER GLANDS

A. *The Pancreas*

The hormone of importance here is insulin. Its relative lack or non-function is the cause of Diabetes Mellitus. The important tests of function are:

i. Blood sugar levels, fasting and 2 hr. p.c.
ii. Blood ketone level
iii. Urinary sugar and ketone levels
iv. The glucose tolerance test (GTT)

Insulin levels can be measured by both an immuno and a biological assay. However, they are used only to diagnose insulin secreting tumours.

(B) *Gonads, Fetal – Placental Unit*

The differentiation of primary and secondary hypogonadism has been dealt with in the discussion of the Pituitary gland. The differentiation of the various adreno-genital syndromes and precociousness is primarily clinical but there are special enzymatic methods available for academic interest.

The Fetal-Placental Unit is the main source of estriol in late pregnancy hence serial measurements of this substance in maternal urine is a good way to follow fetal viability.

(C) *The Parathyroid Gland*

The serum parathormone assay has not yet been perfected. Thus serum calcium levels are the only reliable parameter.

SUMMARY OF THE TESTS

I Thyroid Gland

1. Serum T_4
2. PBI
3. I^{131} Uptake
4. Serum T_3 Uptake
5. TSH Test
6. T_3 Suppression Test
7. Thyroid Scan

II Adrenal Cortex

1. Plasma Cortisone Level
2. Urine Metabolites
   - i. 17-OH Steroids
   - ii. 17 Keto-Steroids
   - iii. 18-Glucuronide
3. ACTH Test
4. Metyrapone Test
5. Dexamethasone Test
6. Plasma Renin Activity
7. Serum Electrolytes

III Adrenal Medulla

1. VMA

IV Pituitary Gland

A. Anterior Pituitary

1. TSH Test
2. ACTH Test
3. GH Level
4. Urine FSH

B. Posterior Pituitary

1. i.v. Saline Test

V Other Glands

A. Pancreas

1. Blood Sugar and Ketones
2. Urine Sugar and Ketones
3. Glucose Tolerance Test
4. Insulin Plasma Levels

B. Gonads and Fetal Placental Unit

1. Urine FSH and LH
2. Urine 17 Keto Steroids
3. Maternal Urinary Estriol
4. Special Enzymatic Tests

C. Parathyroid Gland

1. Serum Calcium level

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Pulmonary Function Tests

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INTRODUCTION

The standard methods in the evaluation of chest disease are history and physical examination, radiological, bacteriological and pathological investigations. In recent years tests have been developed which give quantitative and objective evaluations of pulmonary function.

Pulmonary function tests have contributed much to pulmonary physiology and to our knowledge of the natural course and the effects of pulmonary disease. Clinically, they are a useful diagnostic aid, but they are even more useful in the objective assessment of the prognosis of pulmonary dysfunction and disability. These tests can indicate appropriate therapy for some patients, while sequential determinations can evaluate its effectiveness. Where surgery is contemplated, function tests can give some idea of operative and anaesthetic risks. If the operation involves resection of lung tissue, these tests offer a prediction of the patient's post-operative pulmonary adaptive ability.

By themselves they only indicate how pulmonary function has been altered by a disease process, without regard for the anatomical location, the aetiology or the pathology of the disease. Indeed, if function is undisturbed or minimally altered, the tests will reveal nothing. When used as a supplement to the other methods of investigation however, they can provide valuable information.

TESTS

I Ventilation
  A. Lung Volume: 1. Static 2. Dynamic
  B. Intrapulmonary Gas Mixing
II Diffusion
III Blood Gases IV Exercise

I VENTILATION

A. *LUNG VOLUMES*

1. *Static Lung Volumes*

Determination of the lung volumes does not provide an evaluation of pulmonary function *per se*, but rather an anatomic measurement. This determination is useful because these volumes are altered by disease processes, and also because they provide information about a patient's pulmonary status, which is useful in the assessment of a patient's progress and in the interpretation of other tests.

Vital Capacity

A simple volume recorder, such as a spirometer, can measure the three components of the vital capacity; i.e. the inspiratory reserve volume, the expiratory reserve volume and the tidal volume. Normal breathing inscribes the tidal volume, while maximal inspiration followed by maximal expiration will trace the vital capacity.

Total Lung Capacity

This is the volume of gas contained within the lung at the end of a maximal inspiration. It can be determined from a formula based on vital capacity and age group, or by addition of the measured vital capacity and residual volume (see next section).

Changes in vital capacity and total lung capacity usually occur together and so they are considered together. Vital capacity and total lung capacity vary widely with age, sex, race and stature differences. Average values for a class of individuals may be obtained from tables, but the normal values within each class have a variation of ± 20%. Consequently the absolute values of these volumes are not of much worth unless grossly altered. Of greater importance are the changes in these volumes during the course of some pulmonary disease or in response to therapy.

Reduction in vital capacity and total lung capacity is brought about by an absolute reduction in distensible lung tissue seen in such conditions as atelectasis, bronchial occlusion, pulmonary edema or congestion, pulmonary restrictive disease or surgical excision of pulmonary tissue. Non-pulmonary conditions which decrease the ability to expand the lung also decrease both vital capacity and total lung capacity. These are: (1) limitation to descent of the diaphragm; e.g. ascites, pregnancy, phrenic nerve paralysis; (b) decrease in intrathoracic space; e.g. pneumothorax, pleural effusion; (c) limitation to thoracic expansion; e.g. kyphoscoliosis, thoracic pain due to rib fracture; (d) limitation to respiratory movements from neurological causes; e.g. poliomyelitis, peripheral neuritis, myasthenia gravis.

Functional Residual Capacity and Residual Volume

The functional residual capacity (the volume of gas remaining in the lung at the resting expiratory level) and the residual volume (the volume of gas remaining in the lung after maximal expiration) are usually measured together.

(i) *Open Circuit*

The unknown volume of air within the patient's lung contains about 80% Nitrogen. Continuous breathing of pure O_2 for two to seven minutes displaces the nitrogen into the spirometer. Here, measurement of the total volume of nitrogen allows ready calculation of the original lung volume since all the nitrogen comes originally from the patient's lung.

(ii) *Closed Circuit*

A known volume of helium is added to the spirometer. The spirometer, tubing and lungs make a closed circuit so that rebreathing distributes the helium evenly through this closed system. A sampling of the gas in the spirometer allows the dilution of the helium to be

ascertained. From this the volume of the total system minus the known volume of spirometer plus tubing will give the desired FRC.

Both (i) and (ii) are good determinations because they are almost independent of the pattern of respiration; however, some areas of a patient's lung may be extremely hyperventilated and nitrogen washout or helium distribution does not occur. The test may be continued for longer periods to obtain a more accurate volume. In general the helium method is preferred for ease of performance.

(iii) *Thoracic Gas Volume*

This method requires the use of a body plethysmograph — an airtight box in which the patient sits. This measures the total amount of gas in the thorax, whether it is in communication with the airways or not i.e. it will measure poorly ventilated areas. At end expiration, when the pressure in the alveoli (P) is equal to the almospheric pressure and the lung volume is V, the mouthpiece through which the patient breathes is occluded and the pressure measured. Inspiration causes a new alveolar pressure, P_1 measured at the mouthpiece, and a new lung volume, $V_1 = V + \Diamond V$. ($\Diamond V$ is measured from the pressure change within the atmosphere of the plethysmograph due to the outward expansion of the thorax.) From Boyle's Law.

$$PV = P_1V_1$$

therefore $PV = P_1 (V + \Diamond V)$ and V can be calculated. This method is rapid and accurate. Comparison of (iii) to either (i) or (ii) gives an approximation of the volume of gas in poorly ventilated lung tissue.

An FRC of a certain volume is necessary to ensure a constant alveolar PO_2 so that uneven ventilation and anoxia do not occur. An increased FRC is not necessarily disabling because ventilation of the lung (ability to add as much oxygen as is being removed) is more important than alveolar volume. Yet it is disadvantageous because of the increased muscular effort required to breathe and the limitation on the ability to increase ventilation on demand. Increased FRC is due to (a) structural lung changes, as in emphysema; (b) partial obstruction to an airway, predominantly expiratory, as in asthma; (c) compensatory over-inflation following excision of lung tissue; (d) deformity of the thorax.

Residual volume increase means that the lung is hyperinflated after maximal expiratory effort. This signifies a corresponding reduction in vital capacity and depends on changes in lung tissue, thoracic cage or the respiratory muscles. These changes may or may not be reversible depending on the nature of the disease and may or may not restrict ventilatory ability, depending on the severity. An increase in residual volume is seen in conditions increasing functional residual capacity. A decreased residual volume is seen in diffuse pulmonary restrictive disease and in conditions in which alveolar occlusion occurs in many lung regions.

2. *Dynamic Lung Volume*

These measurements are concerned with the rate of air flow and the amount that is moved. Of importance are the forces bringing about movement of air and the resistances against which they work.

In inspiration work is done by the respiratory muscles to overcome the forces of elastic recoil of lung and thorax, frictional resistance to movement of lung and thorax and the frictional resistance to air flow. On expiration the potential energy stored in the expansion of the elastic structures is utilized to overcome the frictional resistance to air flow and to movement of the lung and thoracic wall.

The dynamic tests performed serve as an index of ventilatory ability and indicate imbalance in the forces previously described. They are most commonly used to detect obstruction to air flow.

Forced Vital Capacity

After breathing normally into a spirometer, the patient is instructed to take a maximal inspiration followed by a maximal expiration. The resulting spirograph tracing inscribes the forced vital capacity (FVC). This tracing can be analyzed by determining the volume expired in the first second (FEV_1) and may be compared to the FVC to give the FEV_1/FVC, which in the normal is greater than 75%. Alternatively, one may draw a tangent to the mid-point of the curve to give the maximum mid-expiratory flow rate (MMFR), which is greater than 3L/second in the normal.

This test is a good index in the presence of obstruction and obstructive lung disease. It does not differentiate between tracheo-bronchial obstruction; e.g. tumour, and obstruction in the smaller conducting airways; e.g. asthma, bronchitis or obstructive emphysema.

Maximum Voluntary Ventilation

This is the maximum volume of gas that can be breathed per minute by voluntary effort. The test consists of rapid deep breathing through a low resistance for 15 seconds and the results are extrapolated to one minute. The test is simple but requires the understanding and cooperation of the patient who may be exhausted by it. The normal values show a variation of 20-30% from the mean, so that interpretation is difficult. Finally, the test is not diagnostic of any condition and may remain normal where gross pathology exists.

Either of the preceding two tests may be repeated after inhalation of a bronchodilator aerosol. This is useful in demonstrating reversibility of the obstructive process. This can be diagnostically useful in differentiating asthma from bronchitis and emphysema, and as a guide to bronchodilator therapy.

B. *INTRAPULMONARY GAS MIXING*

The previous tests of ventilation were concerned with the amount of air moved. A second factor of importance is the distribution of the inhaled air. The tests in this section estimate maldistribution of inspired gases and are based on the premise that hypoventilated areas exchange gas and equilibrate less readily than normally ventilated areas.

(i) *Open Circuit Single Breath*

The patient inspires one breath of O_2 and expires into a spirometer equipped with a continuously recording nitrogen meter. With even distribution the nitrogen concentration will be constant in the expired alveolar air. With mal-distribution nitrogen will be in higher concentration in hypoventilated areas; since these areas empty last in expiration, a marked rise in nitrogen will be seen.

(ii) *Open Circuit Multiple Breath*

The patient breathes oxygen for seven minutes, then expires forcibly. With even distribution this washout will reduce nitrogen in the final alveolar sample to less than 2.5%. If hypoventilated areas exist they will contain a high concentration of nitrogen on forced expiration even after oxygen breathing, assuming the pattern of breathing and FRC are normal.

(iii) *Closed Circuit Multiple Breath*

A known amount of helium in a closed system consisting of lungs and spirometer is rebreathed by the patient until it is distributed evenly in the closed system. With uniform distribution a plotted curve of helium dilution is an exponential function of time, while maldistribution gives a more variable rate. This test is not quantitative but gives an index of gas distribution and can be carried out at the same time FRC is measured.

II DIFFUSION:

The transfer factor (diffusing capacity of lung) for a gas is the rate at which that gas is absorbed from the alveolar gas in ml/minute per mm Hg tension difference between alveous and capillary. It is now thought that $1/Te = 1/Dm - 1/\theta Vc$ where Te is the transfer factor, Dm is the diffusing capacity of the alveolar-capillary membrane, Vc is the volume of blood in the alveolar capillaries in ml and θ is the rate of combination of the gas with the blood.

Diffusion is the process by which a gas moves from the alveolus, through the alveolar-capillary membrane to the plasma. Its rate is dependent upon the physical properties of the membrane (surface area and thickness) upon the physical properties of the gas (solubility and molecular weight) and upon the difference between the tensions of the gas on the two sides of the membrane. The diffusing capacity of the membrane is reduced when there is reduction in the effective surface of gas exchange in the lung, for example aging, parenchymal lung disease with loss of lung tissue, absence of ventilation in parts of the lung. It is also reduced when the thickness of the alveolar capillary membrane is increased. This is not because thickening increases diffusion time *per se* as previously thought (so called capillary block) but is due to a hypoventilation of certain lung areas with a subsequent decrease in the area available for diffusion.

The term $1/\theta Vc$ describes the rate of reaction of the gas with hemoglobin and is the other factor besides diffusing capacity of the membrane that determines transfer factor. The transfer factor is commonly concerned with CO or oxygen; since these gases are not limited by the rate of flow of blood to the lungs, the total volume of blood in the lungs is of prime importance. This factor is responsible for the varying Te seen with exercise and change in body temperature.

Te may be measured for oxygen, the physiologically important gas but the determination is difficult so that carbon monoxide is the gas of choice.

(i) *Single breath*

The subject exhales to his residual volume then inhales a VC breath of test gas containing a small amount of carbon monoxide, helium and oxygen. The breath is held for ten seconds and then expired; an alveolar sample is collected during expiration. The effective volume is obtained by the dilution of helium. Comparing inspiratory and expiratory concentrations gives the dilution of helium in the lungs. The initial concentration of CO is determined by the helium dilution since they are diluted to the same extent. The expiratory concentration is determined by analysis of the alveolar sample.

(ii) *Steady state technique*

The patient breathes a low concentration of CO in a gas mixture until steady state conditions have been reached. The uptake of CO is measured by the analysis of its concentration in inspired and mixed expired gas. The alveolar PCO fluctuates in the respiratory cycle so that use is made of PCO_2 to determine the components of expired gas due to physiological dead space and alveolar ventilation. This requires PCO_2 analysis of arterial blood or of end-expired gas.

The single breath method can be done rapidly and gives the patient no discomfort. Patient co-operation, more equipment and more extensive mathematical treatment of data are required than with the steady state test. The steady state test, on the other hand, is more useful in patients who are exercising or in those who have a reduced transfer capacity. However its main difficulty arises in obtaining a representative sample of alveolar air for analysis.

III BLOOD GASES

The arterial blood gases serve as an overall measure of the lungs ability to perform its function since the continuous process of oxygen removal and carbon-dioxide addition at the tissue level must be balanced

by an equal and opposite exchange with the environment at some other location in the body. Blood is most commonly obtained by arterial puncture and determination of PO_2, PCO_2 and PH are made; oxygen saturation may also be measured.

Blood gas abnormalities pertaining to the lung are due to disturbances of ventilation/perfusion ratio in parts or all of the lung. The ventilation/perfusion ratio may be deranged by changes in the volume of inhaled air or by maldistribution of air or blood to regions of the lung.

(i) Hyperventilation refers to an increase in ventilation beyond that necessary for maintenance of normal arterial blood PO_2 and PCO_2. Little effect is observed on PO_2 since arterial blood is almost saturated under normal conditions; however PCO_2 is lowered and a condition of respiratory alkalosis results. Hyperventilation is commonly seen in anxiety states, CNS lesions, drug ingestion (salicylates), hypermetabolic states (hyperthyroidian fever), metabolic acidosis and mechanical overventilation.

(ii) Hypoventilation occurs in patients in whom respiratory excursion is for some reason inhibited. This may be due to mechanical inhibition of thoracic or lung movement or a neuromuscular disease, diseases effecting neural conduction, neuromuscular transmission or the respiratory muscles themselves. Hypoventilation also occurs in pulmonary disease in which there is functioning lung tissue. The underlying pathophysiology is the same: the volume of inhaled air in the alveoli is inadequate for the metabolic requirements of the body. Consequently, alveolar PO_2 decreases and PCO_2 increases with similar changes in the blood. A condition of respiratory acidosis results.

(iii) Maldistribution of gas occurs with regional changes in elasticity e.g. emphysema; regional partial obstruction of airways, e.g. asthma; combinations of the preceding, e.g. obstructive emphysema; and regional disturbances in expansion, e.g. restrictive disease. Maldistribution of blood occurs in situations where certain areas of pulmonary vasculature are functionally closed by emboli, vascular disease, lung tissue destruction or where shunts exist so that blood passes to the left atrium without perfusing lung tissue; e.g. pulmonary hemangiomas.

IV EXERCISE

For critical assessment, the amount of exercise must be standardized as it is in the Harvard two step test or in the use of bicycle peddling (at a certain speed against a given resistance). This can be useful in:

(a) The clinical assessment of dyspnoea after exertion.

(b) Assessment of pulmonary function reserve: a patient with normal blood gases at rest may show arterial desaturation after exercise due to a low functional reserve.

(c) Differentiation of emphysema and chronic bronchitis: often these two diseases present with a low transfer factor at rest. The use of exercise may overcome the maldistribution present in the bronchitic so that his transfer factor rises while that of the emphysematous patient remains low.

ABNORMALITIES IN TESTS

Two large areas of lung disease have typical patterns of abnormalities in testing.

I *Restrictive Lung Disease*

This area of abnormality is due to processes inhibiting chest wall or lung expansion and to pulmonary disease that decreases lung distensibility. The primary defect is reduction of the lung volumes especially the vital capacity. Compensatory change of respiratory pattern may allow a normal or moderately reduced maximum voluntary ventilation but not so marked as the reduced vital capacity. With increasing severity, the blood gases show a picture of hypoventilation with decreased PO_2, and increased PCO_2. Gas mixing and transfer capacity usually remain within normal limits.

II Obstructive Lung Disease

Obstruction to air flow is greatest during expiration since inspiration tends to distend the airways. In these conditions the forced vital capacity curve is prolonged with a consequent decrease in FEV_1, $FEV_1/FVC\%$, and MMFR. Obstruction by its nature does not allow a compensatory change in respiratory pattern when maximal ventilatory ability is tested, so that the decrease in MVV is most marked. In addition, vital capacity is usually reduced, residual volume increased and maldistribution of gas is observed. Transfer capacity may be reduced where effective area for gas exchange is reduced; this has been used to differentiate emphysema from asthma and bronchitis but may require the use of exercise. The reversibility of obstruction with bronchodilator drugs during measurement of FVC and MVV is most marked in asthma. This is a diagnostic point but this determination can indicate the usefulness of bronchodilator therapy in any obstructive disease.

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Blood Gases Determination

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INTRODUCTION

The complete characterization of a patient's acid-base status via direct pH and P CO_2 determinations on whole blood has become a major function of virtually all hospital clinical chemistry departments. At the Toronto General Hospital in the first six months of 1969, some 8,000 blood gas determinations were performed by the routine laboratory, these included direct P O_2 measurements for further pulmonary gas exchange assessment and monitoring changes due to treatment. The time and money spent for "Blood Gases" now rivals that for the ubiquitous Blood Sugar and BUN determinations, and at its present rate of increase promises to overtake these and other routine tests in consumption of laboratory space and manhours. Much of this expense is a result of the instability of the sample and the lability of the condition which it reflects, as this requires each test be considered STAT and that a trained technologist be on duty 24 hours a day.

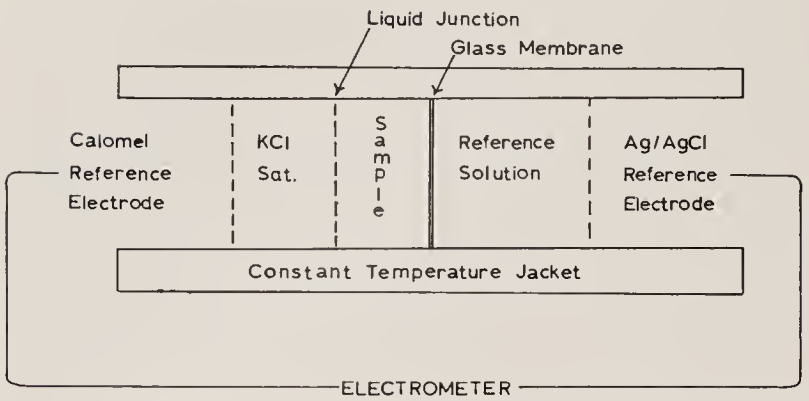
HISTORY OF TECHNIQUES USED

The measurement of pH began in the early 1900's with Sorensen's study of the effect of "acidity" on the level of enzymatic activity. Sorensen noted that there was a difference between total acidity and hydrogen ion concentration, the latter being related to the degree of dissociation of the acids present, and that the concentrations of hydrogen ion was in the order of 10^{-1} to 10^{-7} moles per litre. For convenience Sorensen dropped the minus signs and used the exponents or powers as whole numbers. In French he referred to this Hydrogen power as "puissance Hydrogen", and in 1909 he coined the now familiar symbol, "pH". Sorensen's determinations were first done using colorimetric techniques; later a hydrogen electrode and voltmeter. Measurements of pH especially on whole blood or plasma were extremely difficult, tedious, and time consuming: one measurement might take as long as a day to perform satisfactorily, but surprisingly determinations on "normals" gave values of around pH 7.38, which suggests that we have not really improved on accuracy, but only in the speed and ease of measurement.

In 1922, Walter Hughes of the Harvard Biophysics Laboratories, described the operation of a glass electrode for the measurement of pH. This electrode depended upon the earlier discovery by Haber and Klemensiewicz that there existed a difference in electrical potential across a *thin* glass membrane separating solutions of different hydrogen concentrations. The glass electrode made it very much easier to measure whole blood or plasma pH, as the earlier hydrogen electrode with its catalytic platinized surface was easily "poisoned" or contaminated by protein and other blood components. However, two factors combined to make it all but impossible to measure the relatively small pH differences. First, the voltmeters then in use drew such a great deal of current in making a measurement that they were almost insensitive to small current flow. Secondly, because of the high resistance of the glass membrane, in the order of 30 to 50 megohms, the current flow generated for even large differences in pH was small. This glass electrode did not become practically useful until 1929 when William C. Stadie using the electron tube developed a voltmeter with a sufficiently low current draw that small differences in pH could be measured accurately. His values for arterial blood ranged from pH 7.36 to 7.47. These early bulb-type glass electrodes had additional drawbacks with respect to blood pH determinations. They required a rather large sample, had poor temperature control, and the sample was exposed to the air allowing for loss of carbon dioxide by the sample, giving an erroneous pH.

In 1957, Sanz introduced an anaerobic, capillary glass electrode with precise temperature control for both electrode and sample. The Sanz glass electrode at once made measurement of whole blood and plasma pH rapid and routine. Although many modifications have been produced since then, the basic structure and principle remain the same. Figure 1 shows diagramatically the Sanz electrode. Whole blood or plasma is drawn into the electrode anaerobically using a low vacuum. The hydrogen ions in the sample are thus exposed to the H^+ sensitive glass membrane which is also in contact with a stable reference solution of known pH. The potential generated across the glass membrane is measured indirectly via two reference electrodes connected to opposite terminals of an electrometer (an electromic voltmeter). A problem which arises here is that when whole blood is used, the red blood cells create their own potential at the liquid junction between the sample and the saturated KC1, equivalent to 0.01 pH. Thus, the measured blood pH is too low, but correctable. Although the electrometer actually measures E.M.F. (electromotive force) or voltage, its scale can be calibrated to read directly in pH units. Since the resistance of the glass membrane differs from one electrode to another it is always necessary to calibrate the electrometer with respect to both range and exact reading. This calibration is commonly done using two specially prepared National Bureau of Standards buffers of pH 6.840 and 7.382 measured at 37°C ± 0.1. The buffer of pH 7.382 is also used for checking the calibrations just before or just after an unknown sample is measured. Using the above apparatus and calibration techniques, the Toronto General Hospital routine laboratory shows an overall standard deviation of pH ± 0.007 including both "within day" and "between day" variations.

FIGURE 1: pH Glass Membrane Electrode



The modern history of blood carbon dioxide measurements is generally considered to have begun with Van Slyke (1917) and Van Slyke and Neill (1924), who developed the original method for the determination of total plasma CO_2 using venous blood. Although the technique was complex and time consuming it was reliable and accurate. Natelson in 1951 devised a rather simple apparatus for carrying out a Van Slyke total plasma CO_2 determination in much less time. The Natelson gasometricmanometer consists of an extraction chamber attached directly to a gas burette calibrated for constant volume, a mercury reservoir, and a mercury monometer, which measures the pressure of gas (es) in the burette. A sample of venous blood is taken and centrifuged anaerobically and an accurately measured aliquot of the plasma is introduced into the gas burette. If whole blood is used the accuracy suffers as a result of the wider variations encountered in pipetting whole blood. A known volume of lactic acid is added to the sample of plasma and the mercury column of the gas burette is allowed to fall, thereby creating a vacuum above the mixture. The combination of vacuum and excess strong acid (in this case lactic acid) forces the following reaction to the right:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2\,(d) \rightleftharpoons CO_2\,(g)$$

Essentially the bicarbonate, carbonic acid, and dissolved CO_2 present in the original plasma sample are all converted to gaseous carbon dioxide, $CO_2(g)$ which along with other extractable gases present, i.e. H_2O vap., O_2, and N_2, is reduced to a known constant volume in the gas burette and the total pressure of the gas mixture is measured via the attached manometer. At this point a carefully measured amount of strong base, sodium hydroxide, is introduced anaerobically to force the following reaction to the right:

$$2OH^- + CO_2 \rightleftharpoons CO_3^= + H_2O$$

with the result that all of the gaseous CO_2 is converted to carbonate. There is thus a decrease in the measured gas pressure. This pressure change corrected if need be, for temperature, is converted directly into total CO_2 content in millimoles per litre. It was because of this accuracy and reproducability that the method remained unchallenged until a Polio epidemic at Copenhagen, Denmark in 1952 provided the impetus for change.

Many were dying because all available artificial respirators were already in use. To alleviate the situation medical students were placed on 24-hour duty to operate manual rubber bag respirators. Optimally the patient's P CO_2 was needed immediately at the bedside in order to know when to start and stop the ventilation. However the best existing technique for determining carbon dioxide was the Van Slyke which required 15 to 20 minutes for the determination alone, a highly skilled operator, and only gave total plasma CO_2. Plasma PCO_2 could only be calculated if a pH measurement was also made. In short the Van Slyke method, even using the Natelson apparatus, proved totally inadequate for the conditions of the epidemic. At this time Dr. Paul Astrup of Copenhagen began to develop a more suitable method. At first Astrup developed a method which required the pH of two plasma samples separated anaerobically and equillibrated to a normal arterial PCO_2 of 40 mm Hg. at 38°C. Astrup's original method represented an improvement as pH's could be read faster with less training and a smaller sample than the Van Slyke total carbon dioxide. Later in 1958, Astrup working with the Radiometer Corporation of Copenhagen developed what has become known as the Astrup micro-tonometric method using a modification of the Sanz capillary glass electrode for pH measurements. This method also depended on three pH determinations to obtain the actual PCO_2 of the patient's blood and was based on the fact that a variant of Henderson-Hasselbach equation for plasma showed that pH was inversely proportional to PCO_2. For the physiologic range the relations between log PCO_2 and pH is essentially linear, such that, if the pH of the patients whole blood is known for two fixed PCO_2 levels, two points on a graph of log PCO_2 versus pH may be plotted and a straight line drawn joining the two may be drawn again because of the essential linearity of the relation over the physiologic range. The pH on an unaltered sample of the patient's whole blood can then be measured and the corresponding, i.e. actual, PCO_2 can be read directly from the graph. The Astrup technique utilizes a specially designed apparatus with two constant temperature equilibration chambers into each of which is introduced a "micro" sample of the patient's whole blood, of the order of 50 to 100 microliters. The samples are vibrated to form a thin layer in the chambers to facilitate gaseous and temperature equilibrium and are exposed to separate gas streams of two known PCO_2 levels, usually about 20 and 80 mm of Hg. The resulting equilibrated blood is drawn directly from the equilibration chambers into a modified Sanz electrode for pH determination.

With a few clever additions the graph of log PCO_2 versus pH has become the Siggard-Anderson curve nomogram, see figure 2, from which the acid-base parameters: Buffer Base (B.B.), Standard Bicarbonate, and Base Excess (B.E.), can be read. Blood buffer base or more simply buffer base (B.B.) was introduced in 1948 by Singer and Hastings in an attempt to express in a quantitative fashion the amount of fixed acid or base

present in whole blood. Buffer base was defined as the sum of all the buffer bases of whole blood, i.e.

$$(B.B.) = (Buf^-) + (HCO_3^-)$$

where (HCO^-) represents the total concentration of the conjugate base of the bicarbonate buffer system, and (Buf^-) the concentration of all the conjugate bases of the non-bicarbonate buffer systems of whole blood, e.g. proteinate, hemoglobin, phosphates, etc. Table 1 below lists the blood buffer systems and the approximate percentage of the total buffering which they provide.

Table 1

Buffer System		*Approximate % of Whole Blood Buffering*
plasma bicarbonate	35	53% by Bicarbonate Buffering System
erythrocyte bicarbonate	18	
hemoglobin & oxyhemoglobin	35	47% by Non-Bicarbonate Buffering System
plasma proteins	7	
organic phosphates	3	
inorganic phosphates	2	

Buffer base is therefore independent of PCO_2, the respiratory component, but not independent of hemoglobin concentration. It may be seen from table 1 that hemoglobin and oxyhemoglobin account for some 75% of (Buf^-) under normal conditions.

The concept of Base excess (B.E.), was introduced to simplify the visualization of whether a patient's buffer base, (B.B.), was above or below the "normal" buffer base of 48 m Eq/1. A buffer base concentration of greater than 48 m Eq/1 is described as a positive base excess, +B.E., while a buffer base concentration of less than 48 m Eq/1 is described as a negative base excess, – B.E., both of which are quantitatively expressed as the number of m Eq/1 of buffer base below or above the "normal" value of 48. Siggard - Anderson described the buffer base (B.B.) and base excess (B.E.) curves as the geometric loci for corresponding values of pH and PCO_2 of blood samples which fulfill the equation:

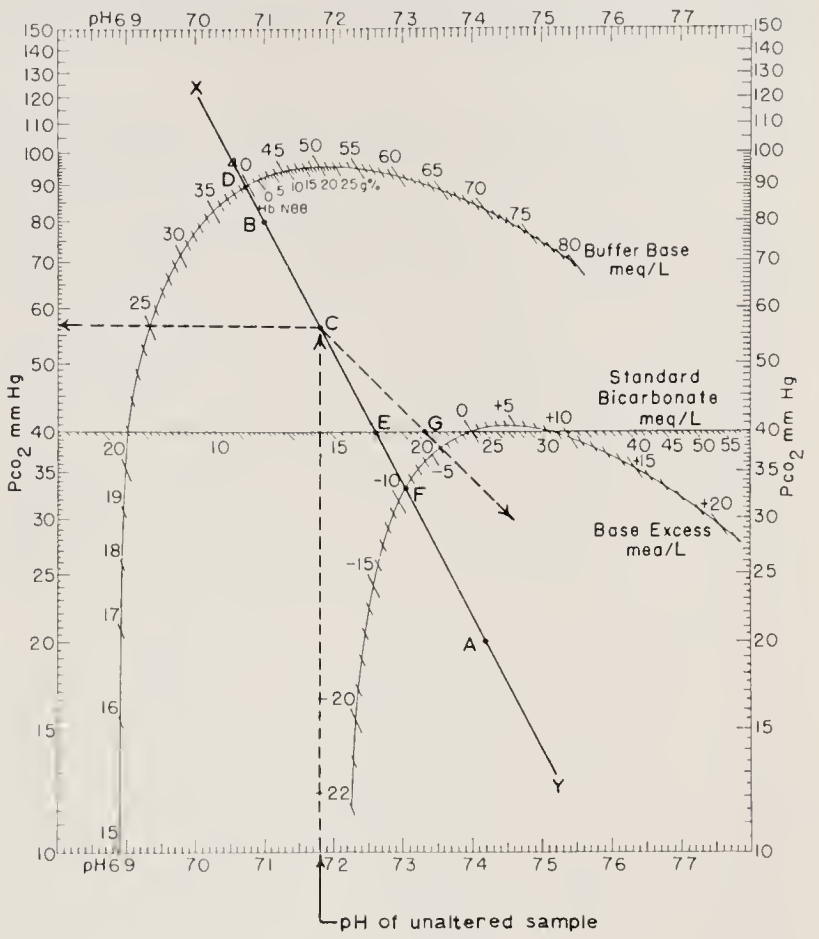
$$BB(cells) - BB(plasma) = K, \text{a constant}$$

where K = O for buffer base curve and K = 12.5 for the base excess curve.

Standard bicarbonate is defined as the bicarbonate concentration in plasma of whole blood which has been equilibrated with a gas mixture with a PCO_2 of 40 mm Hg and sufficient PO_2 to have fully saturated hemoglobin.

It should be noted here that Astrup's equilibration technique requires that $CO_2 - O_2$ gas mixtures be used in equilibration. For if the blood is not fully oxygenated it would be more basic, as reduced hemoglobin is more basic than oxyhemoglobin and the pH/log PCO_2 curve would be slightly displaced to the right.

FIGURE 2: Siggard-Andersen Curve Nomogram



The use of the curve nomogram can be illustrated using figure 2. Points A and B are obtained from the pH measurements on the two blood samples equilibrated to two known levels of PCO_2, i.e. 20 and 80 mm Hg respectively. Line XY is drawn through A and B. Point C represents the point of intersection (measured pH of the unaltered blood sample as it comes from the patient). The value for the patients PCO_2 is then read off from the ordinate graph of the (57 mm Hg). The intersection of line XY with Buffer Base Line at D (39.5 m Eq/l.), the standard bicarbonate line of E (17.5 m Eq/l.), and the base excess line at F (–9 m Eq/l.) permits the direct readout of these parameters. Actual plasma (HCO^-_3) is determined by drawing a line from C at 45 degrees with the standard bicarbonate line. Its intersection with the standard bicarbonate line at G (20.5 m Eq/l.) is the actual plasma (HCO^-_3).

In addition the curve nomogram offers a check on the validity of the Astrup micro-tonometric method, when it is recalled that the normal buffer base (N.B.B.), of a sample of whole blood is defined as the sum of all conjugate bases of whole blood, when the acid-base status is "normal", i.e. (N.B.B.) = (observed buffer base) — (base excess) where (B.B.) = (Buf-) + (HCO_3^-). Since haemoglobin and oxyhemoglobin are the most important members of the non-bicarbonate buffer system represented by (Buf^-), it follows that the concentration of normal buffer base is proportional to hemoglobin concentration. Thus, a subscale is included on the buffer base curve of the Siggard-Anderson curve nomogram which gives the (Hb) for the observed value

of normal buffer base. This value obtained from the graph may be compared to the (Hb) of the sample which has been directly measured.

The Astrup micro-tonometric method was used in the routine clinical chemistry laboratory at the Toronto General Hospital up to October 1966. It required duplicate determinations and at least ½ hour of analytic time per sample. At this time the "direct-read" PCO_2 electrode was introduced. This method required a smaller sample, no duplicates, an analytic time of only 5 to 6 minutes, and allowed for standardization with analyzed compressed gases alone. In addition, tests of precision on paired blood samples over the range 23-133 mm Hg showed that the standard deviation between members of the pair was ±1.20 for the Astrup micro-tonometric method (based on plotted PCO_2 values) and ±0.72 for the Direct Reading PCO_2 electrode.

The principle of the direct reading PCO_2 electrode was discovered by Stow (1957). The direct PCO_2 electrode is essentially the same as the glass pH electrode described earlier. Here the sample is separated from the H^+ sensitive glass membrane by a thin semipermeable membrane, usually teflon, and a bicarbonate solution of known composition. The membrane is freely permeable to dissolved carbon dioxide but not permeable to bicarbonate ions. Thus, dissolved CO_2 from the sample diffuses across the membrane into the bicarbonate solution where it reacts according to the reaction:

$$CO_2(d) + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

to produce a change in the pH of the bicarbonate solution.

Some operators, interested in reducing the "response time" of the electrode, have included carbonic anhydrase, (1 mg. per ml.) in the bicarbonate solution, while others have used hemolyzed fresh blood (0.1 ml. per ml.).

The change in pH is measured as a change in electrical properties of the system, i.e. the conductance of the cell. The output of the electrode can be described by a form of the Henderson-Hasselbach equation:

$$\log PCO_2 = C - pH$$

$$\text{where the constant } C = pK + \frac{\log (HCO_3^-)}{S}$$

Thus for every ten fold change in PCO_2, a change of 1 pH unit results. The (HCO_3^-) can be considered constant here, because it is contained in the reference solution. The output voltage of the cell is directly proportional to the pH and therefore, via the above equation, to a log function of PCO_2. As the electrode is sensitive to the absolute value of PCO_2 it responds identically to samples of blood, gas, or water. Thus permitting the use of humidified, precisely analyzed, compressed gas mixtures for calibrating the electrode for both range and exact reading. In addition to periodic calibration for small changes in the various electrical parameters, each new electrode must be calibrated separately because of individual differences in the resistance characteristics of its particular glass membrane.

Using both the Sanz pH electrode and the "direct-read" PCO_2 electrode with the well-known Siggard-Anderson alignment nomogram, the total CO_2 and (HCO_3^-) may be obtained in a matter of 5 or 6 minutes. The Base excess may also be read if an independent Hb concentration performed on the sample. Supposing that for a particular sample a pH of 7.39 and a PCO_2 of 48 mm Hg were obtained, a line drawn through these values gives a total CO_2 of 30 mMol/l., and an actual plasma bicarbonate of 28.5 m Eq/l. If the measured Hb was 15 g/100 ml., the base excess would be + 3 m Eq/l. However it may be noted that relatively wide changes in (Hb) have relatively little effect on base excess, i.e. for a (Hb) of 10 g/100ml. (BE) is 3.2 m Eq/l. Therefore under most conditions it is both useful and convenient to assume a (Hb) of 15 g/100 l. if the actual (Hb) is not known. The reader is referred to the Siggard-Anderson monogram to verify these facts.

In general a straight line joining any two known variables may be used to compute the other variables and although the alignment nomogram is set for 37°C, it may be used for other temperatures by the addition of a correction factor to the measured pH.

It may be of interest to some that a survey made in 1965 by Logan showed that of all Canadian clinical chemistry laboratories reporting 16% still used Van Slyke, 28% Natelson apparatus, and 45% the Astrup.

MEASUREMENT OF BLOOD OXYGEN CONTENT

The amount of oxygen in the blood may be determined as PO_2, the partial pressure of oxygen, or by oxygen saturation. Oxygen saturation is defined as the oxygen content, i.e., the number of ml. of oxygen per 100ml. of whole blood, divided by the oxygen capacity of whole blood, i.e. oxygen content when blood is fully saturated with oxygen. For example fully saturated blood with Hb of 14.7 gm/100ml. can carry 20 ml. of O_2 per 100ml. whole blood. Therefore its oxygen capacity is 20 ml./100ml. Thus if this blood were found to have an oxygen content of only 10 ml./100ml., its oxygen saturation would be 50%.

There are two fundamental ways of measuring oxygen saturation. One is gasometrically, classically using the monometric Van Slyke method, or the other is spectrophotometrically. Many physiologists consider the Van Slyke as *the* standard reference method, but spectrophotometric methods are theoretically and practically superior.

The partial pressure of oxygen can be measured in three ways. It can be measured with a PO_2 electrode, to within ±2 mmHg, or directly by the Riley bubble technique, to within ±5% of measured value; or, PO_2 can be estimated from a knowledge of oxygen saturation, temperature, and pH.

When PO_2 is high, the direct measurement of PO_2 is a more sensitive indicator of change than is oxygen saturation. On the other hand, when saturation is low, measurement of saturation is a more sensitive indicator of change than PO_2. However in this present article we will restrict our discussion to the direct PO_2 electrode method as it is presently the method of choice for most routine laboratories.

In 1956, Dr. Leland C. Clark, Jr. introduced the membrane covered polarographic oxygen electrode which overcame the problem of protein contamination which had previously made the polarographic method unsuitable for use in biological systems. The Clark electrode measures the changes in current flow through an electrolyte solution. Dissolved O_2 diffuses through the semi-permeable membrane (polypropylene) into a very thin layer of KC1 solution containing a phosphate buffer to neutralize the small effect of PCO_2 on the response. The KC1 soluton is in direct contact with a silver/silver chloride electrode, the anode and a platinum cathode. Application of a polarizing voltage of about –0.7 volts to the platinum electrode results in the following reduction reaction:

$$O_2(d) + 2H^+ + 2e^- \longrightarrow H_2O_2$$

The flow of current is directly proportional to the "availability" of O_2 molecules at the platinum surface and is linear ± 1% from zero to 100 percent O_2. The scale of the electrometer is calibrated to read PO_2 directly in mm. of Hg. using analyzed compressed gases at two levels of PO_2, usually 0 mm Hg and about 150 mm Hg.

All of these direct determinations require a very small sample, i.e. about 30 to 50 microliters of whole blood or plasma; however, because of "memory" or residual which remains to various extents in the electrodes after calibration or calibration check, several times this volume is required to "wash" the electrodes to obtain accurate equilibration for the clinical sample. It is important to remember that the measurements are made with the samples equilibrated to 38°C. Therefore reported values should be corrected for any differences between these temperatures and that of the patient at the time of sample acquisition. This correction has been facilitated by the introduction of several line nomograms which are freely available.

PROGNOSTIC VIEW

The measurement of "blood gases": pH, PCO_2, and PO_2, by rapid reliable instrumentation nomographic techniques has placed the clinical management of patients with virtually any sort of pulmonary or acid-base dysfunction on a firm objective footing for the first time. The increasing awareness by the medical profession of this situation has resulted in a steadily increasing demand for blood gas determinations.

To meet this demand newer equipment is being introduced using a still smaller sample and digital readout. At the Hospital for Sick Children in Toronto the blood gas instruments are now synchronised with a computer which reads the values from the instruments and prints them directly on the requisition. In future the computer will be able to print out results directly on the ward where the sample was taken along with the values of other derived acid-base perameters. There are many workers in the acid-base field, Gambino, et al, who are strong advocating the use of arterialized capillary blood, i.e. blood from a capillary bed which has been gently warmed prior to sampling, or properly drawn venous blood. It has been shown by several investigators that arterial-venous pH differences range between 0.01 and 0.03 pH units, and that this was true even in severe congestive heart failure except for a few cases with differences of 0.08 pH units. There is also an attempt to introduce the reporting of nanoequivalents of hydrogen instead of pH, and the use of the Kintner nomogram. This was introduced by Dr. Kintner in 1967 to make it even easier to visualize the type and degree of abnormality and easier to predict the effects and interrelationship of metabolic and respiratory treatment.

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(More references obtainable from the author)

Blood Groups And Their Clinical Importance

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A transfusion of red cells is therapeutically successful only if the cells survive normally in the recipient's circulation. Since decreased survival of the donor's cells is usually due to antibodies in the recipient's plasma, it is important for the clinician to understand the rationale of the "compatibility" tests which must precede every transfusion. Rapid destruction of transfused red cells may endanger the patient's life; therefore the importance of prompt recognition and adequate investigation of haemolytic transfusion reactions cannot be overemphasized.

RED CELL ANTIGENS AND ANTIBODIES

The term "blood group system" describes the presence of at least two closely related red cell antigens whose formation is determined by the action of allelic genes. In general, blood group genes are neither dominant nor recessive in character but are best described as co-dominant, implying that the effects of a given gene may be recognized independently of the effects of its allele. With the exception of *Xg* on the X-chromosome, all blood group genes are autosomal.

The red cell antigens of the different blood group systems vary greatly in their ability to stimulate the formation of antibodies. By comparing the incidence of two different antibodies with the frequency of opportunity for immunization by their respective antigens, an indication of the difference in "antigenic strength" between these two antigens can be obtained. Differences in strength must be considered when assessing the clinical importance of antibodies which may cause haemolytic disease of the newborn (HDN) and haemolytic transfusion reactions (HTR).

Blood group antibodies can be divided into two main categories:

(i) "naturally occurring" (usually IgM) antibodies which may be the result of immunization early in neonatal life to antigens so widely distributed in nature that exposure to them soon after birth is inevitable, but which, from an empirical viewpoint are classified as such if found in subjects without a history of transfusion, pregnancy or bacterial immunization.

(ii) "immune" (usually IgG) antibodies resulting from a known antigenic stimulus such as blood transfusion, pregnancy or injections containing blood group substances.

However, antibodies of any given antigenic specificity may be composed of more than one type of immunoglobulin molecule and some antibodies classified as "naturally occurring" have been found to be IgG while some classified as "immune" may be IgM.

Serologically these two types behave differently in that "naturally occurring" antibodies are usually said to be "complete" or "saline agglutinins" i.e., they agglutinate red cells (possessing the specific corresponding antigen) when those cells are suspended in saline; they usually agglutinate best at room temperature. "Immune" antibodies are usually "incomplete"; this term implies that the antibodies do not agglutinate saline-suspended red cells. However, it is possible to detect incomplete antibodies by other techniques, such as suspending the cells in 20% albumin, or by the indirect antiglobulin test (Coombs test) described below.

BLOOD GROUP SYSTEMS

At present, 12 main blood group systems have been recognized: ABO, Rh, MNSs, P, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, I and Xg (see Table 5).

ABO: There are four alleles at the ABO locus; A_1, A_2, *B* and *O*. In addition virtually all subjects possess the *H* gene which is responsible for the production of the precursor H substance upon which the *A* and *B* genes act to produce A and B substances respectively. The *O* gene is an inactive allele or "amorph" and therefore the red cells of group O people possess only H antigen on their surface. As shown in Table 1, antibodies are almost always present in the serum of individuals whose red cells lack the corresponding antigens of the ABO system.

Anti-A and anti-B are the commonest cause of transfusion fatalities (see later) and may cause HDN.

TABLE 1
ABO Blood Group System

Phenotype	*Genotype*	*Red Cell Antigens*	*Serum Antibodies*
A_1*	*A_1 A_1* *A_1 O* *A_1 A_2*	A, A_1	anti-B
A_2	*A_2 O* *A_2 A_2*	A	anti-B (1% have anti-A_1)
B	*B B* *B O*	B	anti-A, anti-A_1
A_1 B	*A_1 B*	A, A_1, B	none
A_2 B	*A_2 B*	A, B	(25% have anti-A_1)
O	*O O*	H	anti-A, anti-A_1, anti-B

* Approximately 85% of group A people are sub-group A_1.

Rhesus (Rh): For most purposes, it is sufficient to categorize people as "Rh-positive" or "Rh-negative". This distinction is made by testing the red cells with the commonest Rh antibody, anti-D.

However, the Rh system is quite complex and although a simple division is suitable for most clinical purposes, awareness of Rh antibodies other than anti-D is important. According to the Fisher-Race concept, the inheritance of the main Rh antigens is determined by three pairs of alleles which are genetically closely linked (i.e., at loci which are close together on the same chromosome). A person inherits a set of three Rh genes from each of his parents: *C* or *c*, *D* or *d*, and *E* or *e*. For example when a person inherits *CDe* from his mother and *cde* from his father, his Rh genotype is then described as *CDe/cde*. The antigens are recognizable by the antibodies anti-C, anti-c, anti-D, anti-E and anti-e.

Like the ABO system, the Rh system is of great clinical importance. Unlike the ABO system, the antibodies do not occur regularly but they are very readily stimulated. For example, a single transfusion of Rh-positive blood immunizes at least 50% of Rh-negative *(cde/cde)* recipients. Such recipients are likely to form anti-C as well as anti-D, since most Rh-positive donors are *CDe/cde* or *CDe/CDe*. It must be remembered that Rh-positive (D-positive) recipients are also capable of forming antibodies to antigens within the Rh system. For example, a *CDe/CDe* patient receiving blood from a *cDE/cde* donor may form anti-c, which can cause HTR and HDN.

It is particularly important to avoid sensitizing Rh-negative females of any age, since anti-D is the commonest cause of HDN. A transfusion of D-positive blood in childhood may cause the death of a woman's first Rh-positive infant, even twenty years later. It now seems likely that all cases of primary Rh-immunization, due to the passage of foetal cells during pregnancy, can be prevented by injections of "Rh Immune Globulin" (potent anti-D) to recently delivered Rh-negative women. HDN due to anti-D could thus be eventually eliminated if Rh-negative females *never* received transfusions of Rh - positive blood.

MNSs: The main antigens of this system are determined by allelic genes *M* and *N* and allelic genes *S* and *s*. Combinations of MN and Ss are inherited as units, i.e., *MS*, *Ms*, *NS* and *Ns*. Thus an MN person may be *MS/NS*, *MS/Ns*, *Ms/Ns* or *Ms/NS*. Among Caucasians approximately 28% are type M, 50% are type MN and 22% are type N. The antibodies in this system have been reported only rarely as causes of HTR or HDN.

P: There are three alleles at this locus: P_1, P_2, and an inactive gene *p*.

Anti-P_1 is a common iso-antibody in P_2 subjects; it is always an IgM globulin and thus does not cause HDN (since IgM globulins do not cross the placenta). It usually reacts best at 4°C. However, rare examples which are active at 37°C may cause the destruction of P_1-positive red cells in P_2 recipients.

TABLE 2
P Blood Group System

Phenotype	*Genotype*	*Red Cell Antigens*	*Frequency**
P_1	$P_1\ P_1$ $P_1\ P_2$ $P_1\ p$	P_1, P	80%
P_2	$P_2\ P_2$ $P_2\ p$	P	20%
p	*pp*	none	rare

* In this Table and subsequent Tables, the approximate frequency in Caucasians is given.

Lutheran: The main antigens of this system are determined by a pair of co-dominant genes, Lu^a and Lu^b.

Phenotype	*Genotype*	*Frequency*
Lu(a+b–)	$Lu^a\ Lu^a$	1%
Lu(a+b+)	$Lu^a\ Lu^b$	8%
Lu(a–b+)	$Lu^b\ Lu^b$	91%

The phenotype Lu(a-b-) occurs rarely and, unlike other "minus-minus" phenotypes, may be produced by a dominant suppressor gene which inhibits the action of the Lu^a and Lu^b genes.

Anti-Lu^a and anti-Lu^b are found very rarely and are thus of little clinical importance.

Kell: The main antigens of this system are determined by a pair of co-dominant genes, *K* and *k*. The genotype frequencies are *KK* < 1%, *Kk* 9% and *kk* 90%.

Outside the ABO and Rh systems, anti-K is the commonest immune antibody. It is formed relatively frequently as a result of transfusion or pregnancy and can cause HTR or HDN.

Lewis: The Lewis substances are not primarily red cell antigens but are antigens of the body fluids such as saliva and plasma, and are only secondarily adsorbed onto the red cells from the plasma. The *Le* gene determines the presence of "Lewis a" (Le^a) and "Lewis b" (Le^b) antigens in the body fluids, through a complicated interaction with the *Hh* and *Se se* genes. (Persons of genotype *SeSe* or *Sese* secrete ABH substances in their saliva.) Persons who are homozygous for the inactive gene *le* do not produce Lewis substances and are therefore Le(a-b-).

HTR due to Lewis antibodies are comparatively rare since the Lewis substances present in the donor's plasma usually neutralize the recipient's anti-Le^a and anti-Le^b. Furthermore, within a few days of transfusion the donor's red cells assume the Lewis phenotype of the recipient.

TABLE 4: Lewis Blood Group System

Genotype		*Blood group substances in secretions*	*RBC phenotype*	*Frequency*
Lewis	*Secretor*			
Le/Le or *Le/le*	*Se/Se* or *Se/se*	ABH, Le^a, Le^b	Le(a–b+)	70%
Le/Le or *Le/le*	*se/se*	Le^a	Le(a+b–)	25%
le/le	*Se/Se* or *Se/se*	ABH	Le(a–b–)	5%
le/le	*se/se*	none		

Duffy: The main antigens of this system are determined by a pair of co-dominant genes, Fy^a and Fy^b. The red cells of almost all Caucasians react with anti-Fy^a and/or anti-Fy^b i.e., their phenotype is Fy(a+b-), Fy(a+b+) or Fy(a-b+). The latter phenotype, for example, occurs in people of genotypes Fy^b Fy^b or Fy^b *Fy*. Homozygosity for this "silent" gene *Fy* best explains the phenotype Fy(a-b-) which although found in < 1% of Caucasians is found very commonly among African Negroes.

Anti-Fy^a is formed relatively commonly as a result of transfusion and can cause HTR and HDN.

Kidd: The two main antigens of the Kidd system are determined by two genes Jk^a and Jk^b. The rare subjects who are Jk(a-b-) are thought to be homozygous for a "silent" gene, *Jk*.

Although anti-Jk^a and anti-Jk^b occur rarely, they can cause very rapid red cell destruction and severe transfusion reactions. They are IgG globulins and thus can cause HDN.

Other Blood Group Systems are sometimes associated with HTR and HDN. (See Table 5)

In summary, the two most important blood group systems to be considered in transfusion are the ABO and the Rh systems, the former because of the high frequency of "naturally occurring" antibodies and the latter because of the potency of the D antigen. Following, but by no means comparable in importance to the ABO or Rh systems are the Kell, Duffy, and Kidd systems.

THE SELECTION OF COMPATIBLE BLOOD

In routine laboratory practice, after the recipient's ABO and Rh (D) types have been determined, donor blood of the same ABO and Rh(D) type is selected. Although such selection greatly diminishes the chance of incompatibility, antibodies other than anti-A, anti-B and anti-D are present in the serum of approximately 1% of hospital patients, and therefore routine matching tests of donor's red cells against recipient's serum must be performed to detect these other antibodies.

The compatibility of the donor's cells with the patient's serum is tested by two techniques: agglutination in a saline medium and by the indirect antiglobulin (Coombs) test. Routinely, the cells and serum are incubated together for one hour before the tests are interpreted.

TABLE 5

Human Blood Group Systems*

Blood Group System	*Main Antigens* (on red cells)	*Main Antibodies most frequent examples*	***Naturally occurring*
ABO	A, B	anti-A) anti-B)	always
Rh	C,c,D,E,e	anti-D) anti-c) anti-E) anti-e)	no
MNSs	M,N,S,s	anti-M) anti-N)	yes
		anti-S) anti-s)	no
P	P_1	anti-P_1	yes
Lutheran	Lu^a, Lu^b	anti-Lu^a) anti-Lu^b)	no
Kell	K, k	anti-K	no
Lewis	Le^a, Le^b	anti-Le^a) anti-Le^b)	yes
Duffy	Fy^a, Fy^b	anti-Fy^a) anti-Fy^b)	no
Kidd	Jk^a, Jk^b	anti-Jk^a) anti-Jk^b)	no
Diego	Di^a	anti-Di^a	no
Ii	I, i	anti-I	usually
Xg	Xg^a	anti-Xg^a	no

Others: Au^a, Do^a, Sd^a, DBG (Bg antigens), etc.

* Table from the Toronto General Hospital Blood Transfusion Manual, 1969.

** "Naturally occurring" antibodies are those found in patients who have never been transfused or pregnant.

Saline agglutination tests at room temperature detect IgM antibodies such as anti-P_1 and anti-Le^a. This test also provides a further check on the ABO groups of donor and recipient.

Indirect antiglobulin test (Coombs test). Antibodies such as anti-K which fail to agglutinate saline-suspended cells will, however, become attached to their corresponding antigens on the red cell and may be detected as a "coat" of globulin. When globulin-coated cells are mixed with anti-globulin, the cells are agglutinated.

The globulin "coating" may be *gamma* globulin (such as anti-K) or *beta* globulin (complement bound by an IgG or an IgM antibody). Therefore the antiglobulin reagents should contain both anti-gamma globulin and anti-beta globulin.

The indirect antiglobulin test is a two-stage test:

(i) the donor's cells are exposed to the recipient's serum at 37°C for 1 hour, and are then washed to remove unbound globulin;
(ii) the washed cells are mixed with antiglobulin serum.

The test is positive (i.e., the cells are agglutinated) when the donor's cells contain an antigen to which the patient is already immunized.

Preliminary tests on patients anticipating surgery often provide a helpful warning of difficulties which may be encountered in cross-matching. Such tests are recommended for patients likely to require a large volume of blood e.g., in cardiac surgery. Some days before surgery, the patient's serum is tested for the presence of antibodies by using a mixture of red cells (containing all the important blood group antigens) from two or three selected donors. Any antibodies detected are then identified by further tests (see later), which may require several hours or days to complete.

The Blood Transfusion Service can then be given adequate time to collect blood from special donors (e.g., *CDe/CDe* donors for a patient with anti-c in his serum). The selected blood is then likely to be compatible in direct cross-matching tests performed immediately before the patient's surgery.

RED CELL DESTRUCTION

Intravascular Destruction

Haemolytic (complement binding) antibodies such as anti-A can cause rapid intravascular lysis of incompatible group A red cells. The free haemoglobin thus released is removed from the plasma by several mechanisms.

(i) Haptoglobin binds approximately 100 mg. of haemoglobin per 100 ml. of plasma. The haemoglobin-haptoglobin complex is cleared by reticulo-endothelial (R.E.) cells, which then convert the haemoglobin to bilirubin.

(ii) Some of the haemoglobin is oxidized and the haeme released from some of this is bound to albumin as methaemalbumin, which is also cleared by the R.E. cells, and converted to bilirubin.

(iii) Excretion of haemoglobin in the urine occurs usually when the haptoglobin is saturated and the free haemoglobin level in plasma reaches 25 mg./100 ml. Some of the haemoglobin in the glomerular filtrate is absorbed by renal tubular cells and the iron released in subsequent haemoglobin catabolism is stored as haemosiderin. Later, shedding of these cells may produce detectable haemosiderinuria.

The rate of bilirubin excretion by the normal liver is usually only slightly less than the rate of bilirubin delivery to the circulation. Therefore unless the amount, or rate, of red cell destruction is greatly increased or unless liver function is depressed, the elevation of serum bilirubin is usually not sufficient to produce jaundice. The highest level of serum bilirubin is usually reached between 3 and 6 hours following intravascular red cell destruction.

Extravascular Destruction

Antibodies other than anti-A and anti-B usually cause red cell destruction in the R.E. system, particularly in the spleen. Although the serum bilirubin may rise, haemoglobinaemia and haemoglobinuria are rarely observed.

SYMPTOMS AND SIGNS OF HAEMOLYTIC TRANSFUSION REACTIONS

Accompanying Intravascular Haemolysis

These potentially fatal reactions (occurring usually when ABO incompatible blood is transfused), are nearly always due to an error in the identification of the patient, specimen, or unit of blood, rather than to technical failure to detect incompatibility.

Usually soon after the transfusion has begun, the patient may complain of the following: a feeling of heat along the vein into which the blood is being injected, flushing of the face, lumbar pain, constricting chest pain, headache, nausea, dyspnoea ,and faintness (accompanying a fall in blood pressure).

When the reaction is due to a complement-binding antibody such as anti-A, the group A cells are coated with anti-A and complement (C). It is now known that leucocytes are attracted (by chemotaxis) to the C components on the red cells, and then release various amines such as histamine. These amines may be responsible, through their action on the smooth muscles, for the patient's headache, nausea, dyspnoea and hypotension. The pyrogens released from leucocytes may cause rigors and fever.

Haemoglobinaemia, haemoglobinuria and oliguria may occur, followed by renal failure. Jaundice may develop hours later. There may be abnormal post-transfusion bleeding due to a deficiency of platelets, fibrinogen, and labile coagulation factors, which have been consumed in intravascular coagulation initiated by thromboplastic phospholipids released from lysed red cells.

It is important to remember that some or all of these symptoms and signs may be absent or modified in patients who are anaesthetized or under the influence of opiates. The only signs which might then arouse suspicion of a haemolytic reaction are abnormal bleeding and hypotension persisting despite adequate blood replacement.

Most of these features of rapid intravascular haemolysis usually subside within 24-48 hours. Since there is a high mortality associated with renal "shut-down",

prompt recognition and treatment is of the greatest importance.

Accompanying Extravascular Haemolysis

Since haemoglobinaemia rarely occurs, the only manifestations of a transfusion reaction may be slight fever or chills (developing within a few hours following the transfusion), transient jaundice, or failure of the haemoglobin to rise.

Accompanying Delayed Reactions

In patients who have become sensitized by transfusion or pregnancy, the antibody may be very weak and undetected at the time of cross-matching. The "seemingly-compatible" donor cells may survive well initially but may re-stimulate antibody production. The antibody then causes sudden destruction of transfused cells, usually within a week of transfusion.

The clinical features are usually not as severe as those accompanying immediate haemolytic reactions. Jaundice may be the first sign. In severe anaemia there may be reticulocytosis. The direct antiglobulin test (see later) is often positive but becomes negative when all the donor cells are destroyed.

INVESTIGATION OF SUSPECTED HTR

When a transfusion reaction is suspected, the transfusion should be discontinued immediately. The unit of donor blood should be returned to the blood transfusion laboratory, accompanied by specimens from the patient:

(i) 10 ml. of blood is carefully transferred from the syringe to a dry tube and allowed to clot. Special care is necessary to avoid any haemolysis.

(ii) 4 ml. of blood is gently mixed in a tube containing EDTA.

(iii) the first specimen of urine is collected.

Investigation is carried out as follows, to determine whether haemolysis did or did not occur and to determine if the reaction was due to incompatibility or to some other cause.

(i) The ABO and Rh groups of the donor and recipient are re-determined.

(ii) The cross-match is repeated using pre- and post-transfusion serum.

(iii) The direct antiglobulin (Coombs) test is performed on the patient's cells from the pre-transfusion and post-transfusion specimen. Donor red cells which have become coated with antibodies in the recipient's circulation give a positive direct Coombs test (i.e., they are agglutinated by antiglobulin serum). If the test is positive with both pre- and post-transfusion recipient red cells, the transfusion reaction may have been due to an auto-antibody. Transfusion of plasma, incompatible with the recipient's red cells (e.g., the transfusion of potent anti-A plasma to a group A recipient as may occur when group O blood is used as a "universal donor") regularly produces a positive direct antiglobulin test. This may cause difficulty in interpreting the test result.

(iv) When the R.E. system, which removes agglutinated red cells from the circulation, is "saturated", micro-agglutinates may be detected by "rocking" a few drops of the anti-coagulated post-transfusion blood specimen on a glass slide. Red cells coated with antibodies become spherocytic in vivo and can be detected by examining a stained blood film.

(v) The post-transfusion plasma is examined for the presence of free haemoglobin, methaemalbumin, and bilirubin as follows:

(a) at free haemoglobin concentrations of at least 20 mg. per 100 ml. the plasma will appear very faintly pink. Higher concentrations of free haemoglobin produce increasingly deeper (red) colours. Smaller amounts can be detected by laboratory examination using a spectrophotometric technique;

(b) when the plasma contains only traces of free haemoglobin the brown colour of methaemalbumin may be visually detectable. More sensitive techniques for detecting methaemalbumin involve spectroscopic examination of the plasma (alpha band of methaemalbumin lies at 623-624 mu), and treatment of the plasma to convert methaemalbumin to a haemochromogen with a sharply defined absorption band at 558 mu, followed by spectroscopic examination;

(c) when the bilirubin content of the plasma is markedly elevated above the normal range of 0.2-0.8 mg. per 100 ml., the plasma will appear bright yellow. Laboratory determination of the serum bilirubin concentration should be obtained.

(vi) When an appreciable amount of haemoglobin has been liberated into and subsequently cleared from the plasma, the plasma haptoglobin level will be low and estimation of this level may be useful, especially if investigation is delayed. Serum electrophoresis on paper is carried out, after amounts of haemoglobin varying from 30 to 150 mg. per 100 ml. have been added to samples of the patient's serum. When the amount of haemoglobin added exceeds the binding capacity of the haptoglobin, a band representing free haemoglobin appears. The serum haptoglobin level is taken as the maximum amount of haemoglobin that the patient's haptoglobin can bind.

(vii) The post-transfusion urine is examined for haemoglobin. Alkaline urine containing oxyhaemoglobin is red, but usually the urine is acid and contains the reduced derivative methaemoglobin and appears brown. Spectroscopic examination of the urine will identify either pigment since the alpha band of oxyhaemoglobin lies at 578 mu, whereas the alpha band of methaemoglobin lies at 630 mu.

TABLE 7
Red Cell Phenotypes of Six Selected Donors

Donor 1	0	CCDee	MMS	P_1	Lu(a+)	K–	Le(a–b+)	Fy(a+b+)	Jk(a+b+)
Donor 2	0	CcDee	MNss	P_1	Lu(a–)	K+	Le(a–b+)	Fy(a–b+)	Jk(a+b–)
Donor 3	0	ccddee	MNS	P_1	Lu(a–)	K–	Le(a+b–)	Fy(a+b+)	Jk(a+b–)
Donor 4	0	ccDEe	NNss	P_1	Lu(a–)	K–	Le(a–b–)	Fy(a+b–)	Jk(a–b+)
Donor 5	0	ccddee	MNS	P_2	Lu(a–)	K+	Le(a–b+)	Fy(a–b+)	Jk(a–b+)
Donor 6	0	ccDEE	MMss	P_1	Lu(a–)	K–	Le(a–b+)	Fy(a+b+)	Jk(a+b+)

(viii) Examination of the urine for haemosiderin may be useful if investigation is delayed. Haemosiderin particles in renal epithelial cells of the urine sediment are stained blue by potassium ferrocyanide under acidic conditions.

TABLE 6*
Investigation of a Transfusion Reaction

TEST	TIME AFTER TRANSFUSION REACTION				
	IMMEDIATE	1-3 HR	6-12 HR	24 HR	DAYS
Blood					
Haemoglobinaemia	† † † † † † † † † † † † † †				
Agglutinates	† † † † † † † † † † † † † † † † † † † †				
Direct A-G Test	† † † † † † † † † † † † † †				
Bilirubin			† † † † † † † † † † † † † †		
Methaemalbumin			† † † † † † † † † † † †		
Haptoglobin	† † † † † † † † † † † † † †				
Urine					
Haemoglobin	† † † † † † † † † † † † † †				
Haemosiderin					† † †

† † Indicates period during which test is likely to be helpful in the investigation of haemolytic transfusion reactions.

* Adapted from Table prepared by Dr. J. H. Crookston and Dr. W. H. Francombe.

The various investigations employed are summarized in Table 6.

(ix) Any antibodies detected are identified. The in vitro behaviour of the antibody may give a clue to its identity: (a) haemolysins are likely to belong to the ABO or Lewis systems, (b) saline agglutinins may belong to the ABO, Lewis, P, or MNSs systems, (c) incomplete antibodies are likely to belong to the Rh, Kell, Duffy, or Kidd systems. The patient's serum is tested against a carefully selected "panel" of red cell samples whose full blood groups are known. Table 7 shows a small donor panel whose cells would be useful in preliminary tests to detect antibodies commonly involved in HTR (i.e., anti-E, anti-c, anti-K, anti-Fy^a, anti-JK^a, and anti-Le^a).

Thus anti-c would react with all samples except #1; anti-K would react with samples #2 and #5; anti-Fy^a would react with samples except #2 and #5.

When serological investigations fail to implicate an antibody as the cause of a transfusion reaction, other causes should be considered. For example, a febrile reaction may be due to white cell antibodies and a haemolytic reaction may be due to the transfusion of blood rendered non-viable by faulty storage (freezing, heating), outdating, or bacterial contamination.

FURTHER CONSIDERATIONS

Transfusion accidents are sometimes unavoidable in emergency cases where there is not enough time to perform the necessary cross-matching tests. Usually however, even in urgent cases there will be enough time for the laboratory to determine the patient's ABO and Rh group so that unmatched blood of the same group may be issued. Occasionally the physician may decide that there is no time for any laboratory tests and O Rh-negative blood is issued for transfusion. In this case there is a risk that donor iso-antibodies may lyse the recipient's red cells. Such haemolytic reactions are almost always due to the transfusion of anti-A or anti-B, present in the plasma of the group O "universal donor", to group A or group B recipients. This risk can be minimized by removal of most of the plasma before the donor unit is transfused.

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Laboratory Diagnosis Of Arthritis

R. B. Simpson, B.Sc. 7T1

INTRODUCTION

The arthritic disorders are among the commonest conditions that the physician is likely to encounter. If effective treatment is to be instituted, precise diagnosis is essential. As in all clinical medicine, a good history and a thorough physical examination are the basis of diagnosis. However, because there is frequent overlap of symptoms and signs in different diseases of connective tissue origin, routine and special laboratory tests for these diseases are vital for exact diagnosis and are continually increasing in number and sophistication. This review will present a working classification of arthritis based on certain characteristic laboratory and X-ray features of these diseases followed by a brief description of some recent advances in the laboratory investigation of arthritis.

Since recognition of ankylosing spondylitis and osteoarthritis is largely dependent on characteristic clinical and X-ray features rather than on changes in laboratory tests, discussion in this paper will be limited to a consideration of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and gouty arthritis.

CLASSIFICATION

Although arthritis may be inflammatory, metabolic, degenerative, or neoplastic, stated simply, there are four main kinds of arthritis most likely to be encountered. These are RA, ankylosing spondylitis, gout and osteoarthritis. The physician should be completely familiar with these common types of arthritis. In RA a variety of serum autoantibodies are found, the best known of which is called rheumatoid factor; the polyarthritis is symmetrical, affecting small peripheral joints, and cutaneous nodules are occasionally present. Ankylosing spondylitis features sacroilitis and an absence of autoantibodies. Patients with gout have hyperuricemia, crystal synovitis, and occasionally tophi. Lastly, osteoarthritis presents the typical appearance of cartilage loss on x-ray examination. The complete classification below shows the range of diseases involved.

Table 1
CLASSIFICATION OF ARTHRITIS[1]

INFLAMMATORY

Seropositive polyarthritis:
- Rheumatoid Arthritis
- Collagen "Connective Tissue" disorders
  - – Systemic Lupus Erythematosus
  - – Polyartheritis Nodosa
  - – Polymyositis, Dermatomyositis
  - – Systemic Sclerosis (Scleroderma)
- Hypersensitivity disorders
  - – Rheumatic Fever
  - – Serum sickness
- Microbiological
  - – Septic
  - – Tuberculosus
  - – Viral
  - – Fungal

Seronegative polyarthritis:
- Ankylosing Spondylitis
- Reiter's Syndrome
- Psoriatic Arthropathy
- Juvenile Arthritis (Still's Disease)
- Ulcerative Colitis, Regional Enteritis

METABOLIC – *Crystal Induced Synovitis*
- Gout (sodium monourate)
  - – Primary
  - – Secondary (Renal, drug induced, myeloproliferative)
- Pseudogout (Calcium pyrophosphate)

DEGENERATIVE – *Cartilage Loss*
- Primary or Familial Osteoarthritis
- Secondary Osteoarthritis
  - – Post traumatic
  - – Neuropathic (Diabetes, Syphilis, Syringomyelia)
  - – Endocrine (Acromegaly)
  - – Metabolic (Ochronosis)
  - – Haematopoietic (Haemophilia, Hemochromatosis)

NEOPLASTIC
- Villondular Synovitis
- Pulmonary Osteoarthropathy

ROUTINE LABORATORY PROCEDURES

Routine Blood Work

Routine blood work may be helpful in differentiating some arthritic conditions. Mild anaemias often occur in RA and SLE: the former being hypochromic, normocytic with marrow that usually shows adequate or increased iron stores and the latter being a normochromic, normocytic anaemia which may be haemolytic. The white blood count is generally slightly increased in RA as well as serum sickness and rheumatic fever. It is distinctly elevated in polyarteritis nodosa but decreased in SLE and in RA patients with Felty's syndrome. Platelet counts tend to be increased in RA and polyarteritis but are low in Felty's syndrome and SLE.

Routine Urinalysis

Urine examination is important in discovering many of the sequelae of arthritic disorders. Proteinuria and microscopic abnormalities may be the first indication of renal involvement in SLE.

Erythrocyte Sedimentation Rate

This test is a highly sensitive indicator for the presence of inflammation although it does not pinpoint the cause of the inflammation. The changes in blood which correlate with erythrocyte sedimentation rate (ESR) during inflammatory processes are an increase in plasma proteins especially fibrinogen, alpha globulins and gamma globulins[2]. The ESR serves as a monitor of the inflammatory activity and response to treatment in RA and other collagen disorders. The best method of evaluating ESR is the Westergren Method since the scale used in this system measures the higher range of values found in many rheumatic disorders.

Serum Protein Electrophoresis

Changes in the serum protein electrophoretic pattern are commonly found in a variety of collagen diseases. Ogryzlo[3] reported that serum albumin was decreased in 55% of patients with RA and that 37% had elevations of serum gamma globulins. However, normal electrophoretic patterns were found in 25% of these cases. In SLE decreased albumin and increased gamma globulin have been reported in 47% and 50% of cases, respectively[4]. These protein changes are closely correlated with disease activity and may be reversed by steroid therapy.

INVESTIGATION OF RHEUMATOID ARTHRITIS AND THE COLLAGEN DISEASES

Rheumatoid Factor

Rheumatoid factor (RF) has the characteristics of an autoantibody to gamma globulin. The titre of RF may be used to follow the activity and severity of the disease in patients with RA. RF occurs in other conditions which are not likely to be clinically confused with rheumatoid arthritis. The prevelance of RF in such diseases is listed below:

Table 2

Condition	*Prevelance[5] (– percentage of cases with positive RF)*
Classic rheumatoid arthritis Sjogren Syndrome Severe systemic rheumatoid arthritis	virtually 100%
Juvenile rheumatoid arthritis Psoriatic arthritis Arthritis with ulcerative colitis	10%
SLE	5% – 10%
Subacute bacterial endocarditis (SBE)	41%
Pulmonary silicosis	50%
Chronic interstitial pulmonary fibrosis	60%
Chronic infections	5% – 10%
Normal subjects (increases with age)	5%

There are many different kinds of anti-gamma globulin antibodies (Rheumatoid factors) in the serum of patients with rheumatoid arthritis and each has specificity for different antigenic determinants of human immunoglobin G (IgG, which has a Svedberg sedimentation constant of 7S). The entity called rheumatoid factor in the serum of rheumatoid patients has been found to be a complex of IgG and immunoglobin M (IgM, 19S); the complex has a sedimentation constant of 22S. The current concept is that RF's are directed against antigenic determinants which are either created or exposed when the IgG molecule is altered or denatured in some way. The tests for rheumatoid factor use many different assay systems, the commonest being the latex test in which particles of latex are coated with human IgG. In the coating process, the IgG is denatured. A high titre of RF is indicated by agglutination of the latex particles (see figure 1).

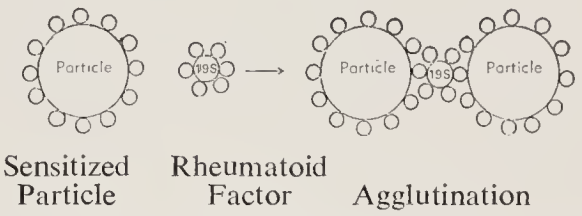

FIGURE 1: Schematic representation of the interaction of rheumatoid factor with a sensitized particle to give agglutination[6].

LE Cell

A distinctive appearing cell in the marrow of patients with SLE was first noted in 1948[7]. The LE cell test developed from this observation and has proved to be the most specific test available for active SLE. If a patient does not have a positive LE cell test, it is unlikely that she has active disease. However, a positive result does not necessarily mean that she has SLE since up to 15% of patients with RA as well as a variety of other disorders have positive LE cell tests. The serum of patients with SLE has been found to contain many antibodies directed toward nuclear material in various kinds of tissues. The LE cell test has been found to be specific for antibodies directed against nucleoprotein. When the serum of SLE patients is incubated with a culture of peripheral leukocytes, a reaction between nucleo-protein antibody and the nuclear material of some white cells causes a change in the staining characteristics of the nuclei. Other leukocytes are attracted to this gammaglobulin-coated material and phagocytosis occurs resulting in the formation of LE cells which can be identified by their morphologically typical inclusions[8].

Anti Nuclear Factor

The anti nuclear factor test or immunofluorescent spot test for antinuclear material was developed in the late 1950's. The test is less specific than the LE test in diagnosing but is a more sensitive indicator of anti-nucleoprotein antibody than the LE cell test.

The test is performed by incubating a substrate of either peripheral blood leukocytes or rat liver slices with the patient's serum. The serum is then flushed off the cells of the substrate with buffer. Rabbit antisera to human gamma globulin which has been conjugated with a fluorescent dye is incubated with the cellular substrate. After a second flushing with buffer, any fluorescent stain remaining indicates the patient's serum contained antinuclear antibodies that were fixed to the substrate cells during the first incubation.

Immunofluorescent Skin Test

It is interesting in relation to the fluorescent technique noted above that Percy & Smyth[9] have recently reported a technique, wherein frozen sections of punch biopsies of normal skin were taken in 150 patients and stained with *fluorescein-conjugated* antiserum directed against IgG, IgM, IgA, transferrin and fibrinogen. Fixation of the antiserum to the dermal epithelial junction was demonstrated in 45 of 50 patients with SLE and 9 of 100 control patients. This approach may prove useful as another sensitive diagnostic test for SLE.

Anti-DNA & Free DNA

Another antinuclear antibody which has recently been discovered is directed against deoxyribonucleic acid (DNA)[8]. It has been found clinically that high anti-DNA levels in patients' serum correlate well with the occurance of acute exacerbations of SLE. As with other antibodies, anti-DNA may be measured in several ways including complement fixation and immunofluorescent tests. Precipitin tests have been used to demonstrate the presence of "free DNA" in the serum of some SLE patients. To detect "free DNA", the method uses a test solution of sera from patients with SLE. The test is not too useful clinically yet since the titre of DNA must be very high to be detected. The possible role of anti-DNA in the pathenogenesis of lupus nephritis has been suggested by 1) the correlation between increasing levels of anti-DNA during the acute phase of the disease and 2) recent evidence that DNA and anti-DNA have been detected by immunofluorescent techniques on the renal glomerular basement membranes of patients with lupus nephritis[11].

Serum Complement

Serum complement levels have been studied recently in relation to many disease of possible immunological origin such as SLE, RA, serum sickness and acute glomerulonephritis. Complement levels (C'H50) have been determined by measurement of the amount of fresh serum required for 50% lysis of sensatized erthyrocytes. Immunochemical identification of individual components has led to the development of techniques for the determination of individual components of the complement system by immunodiffusion in agar[12]. Although the serum level of complement in patients with RA has been shown to be normal or high, synovial fluid levels are low. The sera of SLE patients with active disease are uniformly low.

Summary of the Value of Tests Involved in Monitoring SLE

In a recent study of 96 patients with SLE, Schur and Sandson[13] reported that patients with anti-DNA antibodies or serum complement levels of less than 50 C'H50 units/ml. were more likely to have renal involvement. Very low complement levels and high levels of anti-DNA were always associated with active disease especially active renal disease. A 50% fall in serum complement levels in 22 patients was accompanied by the onset of active nephritis in 19 patients. These immunological parameters will undoubtedly be used increasingly in future to assess disease activity in SLE patients, predict their clinical course, and evaluate their response to therapy.

TESTS FOR GOUT

The serum uric acid is the most important parameter in the diagnosis and management of all forms of gout. The normal value of serum urate for men is in the region of 5.1 ± 1.5 mg/100 ml[14], using the enzymatic method with the values for women being about 1 mg/100 ml lower until after the menopause. The upper limit of normal is 7.0 mg%. These values agree with the theoretical solubility of uric acid in water, 6.4 mg%. Most patients with acute attacks of gout have levels in the range of supersaturation, above 7.0 mg%. The actual onset of an attack seems to vary according to the specific circumstances required to precipitate the crystals in synovial fluid since supersaturated solutions of uric acid are often remarkably stable until some type of seeding phenomenon potentiates the precipitation of crystals[14].

It is of interest that the serum uric acid (SUA) level is decreased by salicylates (4 – 6 gm per 24 hrs), phenylbutazone, uricosurics, allopurinol, and corticosteroids. However, levels of salicylates less than 2 gm per 24 hrs and thiazides increase the SUA. Colchicine, it must be emphasized, does not effect SUA level.

Some 20 to 25 percent of patients with gout are characterized by the over-production of uric acid. The hazard of renal calculi is greater in this group which can be discovered by using a control diet and then measuring the 24-hr excretion rate of urate.

Recent studies[15] involving primary fibroblast cultures originating from skin biopsies as well as results of trials with the xanthine oxidase inhibitor, allopurinol, have shown differences in levels of enzymes involved in purine metabolism. Tests of such enzymes may prove useful in future to classify gout in terms of specific enzyme deficiencies.

SYNOVIAL FLUID

General Approach

Synovial fluid with its dependence on the synovial membranes for its composition can be of great value in the diagnosis of joint effusion. As with all pathological fluids, the laboratory examination involves the fluid's physical, chemical, bacteriological, cytological, and immunological properties. One important biochemical test is the mucin clot test in which a drop of synovial fluid is added to 3% - 5% acetic acid. The nature of the clot which is mostly hyaluronic acid is indicative of the degree of inflammation in the joint. A tight ropy clot is in keeping the intact hyaluronate seen in osteoarthritis whereas the loose fragmented clot of RA is in keeping with depolymerization of hyaluronate.

The crystals found in synovial fluid itself or those crystals ingested by polymorhs can now be easily detected by means of polarizing microscopy which is simple and available in most laboratories. The uric acid crystals of gouty arthritis have a characteristic needle-like appearance whereas calcium pyrophosphate crystals are rhomboidal in shape.

By cytological examination, a white blood count and differential may be made. Cytoplasmic inclusions within polymorphonuclear leukocytes can be discovered with the phase contrast microscope in some synovial fluids. In rheumatoid fluids, if inclusions are found, RF can almost always be demonstrated in these inclusions by immunofluorescent or other techniques. The diagnostic significance of this type of cell called the RA cell or ragocyte is not known as yet.

The immunological tests include measurement of the levels of rheumatoid factor, antinucleoprotein, anti-DNA, or complement levels. Recently,[16] there has been abundant evidence that there is intraarticular utilization of complement in some paitents with rheumatoid arthritis. The level of total haemolytic complement in synovial fluids shows marked reductions especially in those patients with positive serum rheumatoid factor tests.

Studies on individual components of complement point out the possible role of complement in the pathogenesis of synovial inflammation. It is Ruddy & Austen's contention that reductions in individual complement components observed by them in both rheumatoid arthritis and juvenile rheumatoid arthritis resemble those that would be expected from intra-articular fixation and activation of the complement system by an immunological system in a sequence of events consistent with a local inflammatory reaction.

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Student Research

Motor-Nerve Conduction Studies In Children And Adolescents With Diabetes Mellitus

INTRODUCTION

Motor-nerve conduction velocities have long been used to study peripheral nerve function. More recently these determinations have been made on diabetic patients who developed neuropathy.

Clinical neuropathy occurs in 25 to 90% of the adult diabetic population.[1] The first signs of neuropathy are usually sensory – peripheral and symmetrical. Since objective testing for sensory impairment is difficult, nerve conduction studies are valuable when decreased function of the peripheral nervous system is suspected. In adults, the decrease in motor-nerve conduction velocities is proportional to the duration of diabetes, and is further pronounced if clinical neuropathy is present.[1]

In juvenile diabetics, the prevalence of clinical neuropathy has been reported to be from 2 to 25%.[2-4] Since juvenile diabetics are relatively more free from clinical neuropathy, one wonders if the functional state of their peripheral nervous systems remains normal for a longer absolute time. Gamstorp, Khalouf, and Lawrence and Locke reported decreased nerve conduction velocities in juvenile diabetics without clinical neuropathy. Gregersen reports decreased motor-nerve conduction velocity in 18 diabetics aged 15 to 44 years proportional to duration and control of diabetes.[5] This study was begun to determine motor conduction velocities in young diabetics and to examine the effects of duration and control.

MATERIALS AND METHODS

Ulnar and peroneal motor nerve conduction velocities were determined in 37 juvenile diabetics – patients and out-patients at the Hospital for Sick Children. Normal ulnar and peroneal conduction velocities were established in 24 children selected from the head injury patients at the Hospital for Sick Children. These children had no peripheral nerve disorder, no history of diabetes, and were of the same age group as the diabetic patients.

Prior to measuring the nerve conduction velocities, each diabetic patient was examined neurologically. Biceps, brachoradialis, triceps, patellar and ankle reflexes and perception of vibration (128 cps), light touch, and painful stimuli were noted as present or absent. Each patient was asked specifically if he experienced paresthesias – pins and needles and burning sensatoins. Additional information was recorded from the patients' charts: duration of diabetes, dose and type of insulin, fasting blood sugars and urine sugars determined at the clinic, complications, age, height, and weight.

Ulnar and peroneal conduction velocities were usually determined in the right arm and leg: if previous trauma or infection was known to have occurred in a right extremity, the velocities were determined on the left side. Percutaneous electrical stimulation of the ulnar and peroneal nerves was performed in the following manner: the ulnar nerve was supramaximally stimulated by bipolar gold surface electrodes at the elbow. Identical electrode were placed on the skin over the belly of abductor digiti minimi (recording electrode) and at the base of the little finger (reference electrode). With stimulation, the stimulus artifact and muscle action potential were displayed on an oscilloscope screen. A calibrated sweep speed provided the time base. This procedure was repeated with the stimulating electrodes at the wrist. From the oscilloscope sweeps, the latency times were measured – the times required for a *stimulus* to travel (1) from the elbow and (2) from the wrist to elicit *contraction* of abductor digiti minimi. The surface distances between the corresponding points of stimulation and the recording electrode were measured with a tape measure. The motor nerve conduction velocity was determined in meters per second:

l_1 = latency time, stimulating electrodes at elbow
d_1 = distance from stimulating electrodes at elbow to recording electrode over abductor digiti minimi
l_2 = latency time, stimulating electrodes at wrist
d_2 = distance from stimulating electrodes at wrist to recording electrode over abductor digiti minimi

$$\text{Velocity} = \frac{d_1 - d_2 \quad (\text{m})}{l_1 - l_2 \quad (\text{sec})}$$

A similar procedure was used for the peroneal nerve, except that the nerve was stimulated at the knee and ankle with the recording electrode over extensor digitorum brevis.

RESULTS

A. Controls

The 14 males and 10 females ranged in age from 5 to 16 years with an average of 9 years. Mean ulnar and peroneal motor conduction velocities were determined for children (a) less than and (b) greater than 8 years of age (Table 1). The differences between the means are small and insignificant ($\sigma = 0.05$), two tailed t - distribution. On this basis, the nerve conduction velocities in this study are not grouped according to age.

The greater mean peroneal conduction velocity in the 5- to 8-year-old group is an unexpected result. Gamstorp found that peroneal nerve conduction remained

fairly constant and did not increase significantly after the age of 3 years.[2] Since the mean peroneal conduction velocities of the two age groups are not significantly different and the pooled nerve conduction velocity means compare fairly well with published values for adults, the high peroneal conduction velocity may be explained by small sample size. (Thomas and Lambert report that nerve conduction velocities reach normal adult values by age 5 years.[6])

The mean conduction velocities for males and females do not differ significantly.

B. Juvenile Diabetes

The 18 males and 19 females ranged in age from 5 to 18 years with an average of 12 years. At the time diabetes mellitus wias diagnosed, the patients' ages ranged from 1 to 16 years. The known duration of diabetes extended from newly diagnosed (6 patients) to 12 years. Twenty patients took Lente Insulin with an average daily dose of 25 units. Thirteen patients took Lente and Regular Insulin: the average combination dose was 35 units Lente and 9 units Regular. Two patients took Lente-Semi Lente combinations, one took Lente-Ultra Lente, and another, NPH.

The only evidence of neuropathy consisted of absent knee jerks in one patient. Although 22% of the patients admitted to having cramps, cold feet, or some aberrant sensations, the replies were so vague and difficult to interpret that they cannot be used as reliable evidence of neuropathy. Prevalence of clinical neuropathy in juvenile diabetics has been reported as 2 to 25%.[2 4] One patient had diabetic retinopathy.

Serial fasting blood sugars and urine sugars determined at the diabetic clinic were averaged in an attempt to estimate diabetic control. (Caird correlated these values to estimate long term control in adult diabetics.[7]) Correlations for 18 juvenile diabetics were poor and could not be used to estimate control.

Mean ulnar and peroneal nerve conduction velocities were determined for juvenile diabetics whose duration of disease was (a) less than 1 year, (b) 1 to 4 years, and (c) more than 4 years (Table 2).

Table 1 — Motor Nerve Conduction Velocities in Normal Children

Subjects	*No.*	*Ulnar nerve Mean ± s.d.*	*Peroneal nerve Mean ± s.d.*
5- 8 years old:	10	59.0 ± 4.5	57.5 ± 5.0
8-16 years old:	14	59.6 ± 4.5	53.9 ± 4.2
5-16 years old:	24	59.3 ± 5.1	55.4 ± 4.8
Male	14	60.1 ± 5.4	56.9 ± 5.8
Female	10	58.3 ± 2.4	53.2 ± 3.6
15-44 years old: (by Gregersen[5])		59.2 ± 6.2 (29 subjects)	50.8 ± 3.9 (8 subjects)

Table 2 — Motor Nerve Conduction Velocities in Juvenile Diabetics

		Conduction velocity, m/sec	
Subjects	*No.*	*Ulnar nerve Mean ± s.d.*	*Peroneal nerve Mean ± s.d.*
Diabetes for less than 1 year:	15	59.2 ± 4.2	53.2 ± 5.4
Male	9	58.9 ± 5.0	53.1 ± 5.3
Female	6	59.6 ± 4.1	53.4 ± 5.5
Diabetes for more than 1 year but less than 4 years:	14	56.7 ± 6.2	48.6 ± 5.5
Male	6	58.0 ± 5.5	49.8 ± 5.9
Female	8	55.0 ± 6.1	47.7 ± 5.4
Diabetes for more than 4 years:	8	51.5 ± 3.0	44.4 ± 5.0
Male	3	50.7 ± 2.3	41.8 ± 1.6
Female	5	52.0 ± 2.8	46.0 ± 5.4

— Differences between 53.2, 48.6 and 44.4 m/sec are significant.

— 59.2 and 56.7 are not significantly different. 58.0 m/sec (the average of these two mean ulnar conduction velocities) is significantly different from 51.5 m/sec.

The differences between control mean velocities and the mean nerve conduction velocities of diabetics of less than 1 year duration were not significant ($\sigma = 0.05$). The difference between the mean nerve conduction of diabetic patients for less than 1 year and that of diabetics for more than 1 year but less than 4 years was significant for peroneal nerve conduction velocity. The differences between peroneal mean conduction velocity in patients for 1 to 4 years (48.6 ± 54 m/sec) and in patients for more than 4 years was again significant. If the mean ulnar conduction velocity of diabetics for less than 4 years (58.0 m/sec) is compared with the mean value for diabetics for more than 4 years (51.5 m/sec), there is a significant difference.

There were no significant differences in mean nerve conduction due to sex.

These results suggest that motor nerve conduction velocity is reduced with the duration of diabetes. The decrease begins earlier and appears greater for the peroneal nerve: ulnar mean conduction velocity declined 13% (59.2 to 51.5 m/sec) and peroneal, 17% (53.2 to 44.8 m/sec) in patients studied.

Gamstorp and Khalouf have independently reported decreased nerve conduction velocities in juvenile diabetics.[2 3] Although Gregersen also found decreased nerve conduction velocities in 18 diabetics (aged 18 to 44 years), he found that peroneal conduction velocity decreased most significantly during the first year of diabetes and remained virtually unchanged in diabetics of 2 to 4 years duration.[5]

DISCUSSION

The unexpected high peroneal conclusion velocities in normal children less than 8 years of age must be investigated. A small sample size or some part of the procedure may explain the results.

The study of juvenile diabetics revealed a decrease in the mean conduction velocities for the ulnar and peroneal nerves, with the earlier and greater decrease from normal in the peroneal nerve. The velocities reported here are comparable to those reported by Gregersen. In his study of 18 diabetics aged 15 to 44 years, peroneal mean conduction velocity, for diabetics of less than 5 years duration, was 46.7 m/sec (4.1 m/sec below the control mean): it was reduced to 41.8 m/sec in diabetics of 5 to 14 years duration. The mean ulnar conduction velocity was only decreased 2.6 m/sec in diabetics of less than 5 years duration (normal, 59.9 m/sec: diabetics less than 5 years, 57.3 m/sec). In diabetics of more than 15 years duration the mean ulnar conduction velocity was 48.5 m/sec. As mentioned earlier, Gregersen was impressed that "MCV was reduced as early as the first year after onset of diabetics."[5] The results of this study suggest that motor conduction velocity decreases with the duration of diabetes – no significant decrease in ulnar or peroneal conduction velocities was noted in diabetics of less than 1 year. Gregersen never stated the proportion of juvenile and late onset diabetics in his study: this information might explain the discrepancy in conduction velocities. Juvenile diabetics have a relatively abrupt onset of disease while late onset diabetics may be mildly diabetic for years before diagnosis.

The reduced conduction velocities in juvenile diabetics, who are relatively free of clinical neuropathy, suggest that an early and clinically unrecognizable dysfunction of the peripheral nervous system may be present in diabetic patients of any age.

Demyelinization is found in diabetics of long duration with neuropathy.[5] There does not appear to be any histological examination of nerves in relatively short duration juvenile diabetics.

The most important sources of error in motor nerve conduction determinations arise in measuring the latency time and surface distances between electrodes. The use of supramaximal stimuli is extremely important. Temperature differences in patients' perineural tissues is a variable that should be kept in mind.

SUMMARY

Motor nerve conduction velocities were determined on the ulnar and peroneal nerves of 24 young normal children and 37 juvenile diabetics. A comparison of the mean motor conduction velocities of the diabetic patients of less than 1 year duration with those of a normal population revealed that the decrease for the daibetic group was not significant in either nerve. The peroneal mean conduction velocity was decreased significantly in the patients of 1 to 4 years duration: it had again significantly decreased in diabetics of more than 4 years duration. In diabetics of 1 to 4 years duration, ulnar mean conduction velocity was not significantly different from the normal mean. The mean ulnar conduction velocity of diabetics for more than 4 years was significantly lower than that of all diabetics of less than 4 years duration. Nerve conduction velocity decreased with duration of diabetics . . . in the absence of clinical neuropathy.

No significant differences in conduction velocity were found due to sex.

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Clinicopathological Conference

Heart Failure After 30 Years of Dyspnoea

CASE PRESENTATION

Mr. H. H. Age 75 years.
Final Admission: July 21st, 1964.
Died: Sept. 2, 1964

Mr. H. H. first presented in December 1959 with a three-week history of wheezing, shortness of breath and decrease in exercise tolerance. He responded well to bronchodilators and antibiotics.

These same symptoms had been present in increasing intensity for 29 years and had necessitated his retirement from hard-rock mining in 1930 after six years in this employment. Since then the symptoms were repeatedly aggravated by frequent respiratory infections. He had numerous hospital admissions for injuries, disc disease, myocardial infarction (1948), excision of a tumour (type unknown) from mouth, pneumonia and his present symptoms.

He was next admitted in March 1963 with a month history of almost continuous dyspnoea, even at rest, with associated wheezing, white to grey sputum, and swelling of his ankles. Examination at that time revealed a well-developed man in respiratory distress, using accessory muscles of respiration, and wheezing. He showed bilaterally equal chest movement, with diminished air entry. Jugular venous distension (JVD) was 3 - 4 cm., apex rate 96 per minute and regular, blood pressure 160/94, apex beat – 5th interspace at mid clavicular line; no murmurs or abnormal sounds were heard. The liver was palpable 3 cm. below the costal margin and he had pitting edema of both ankles. Chest x-ray showed diffuse nodular pulmonary fibrosis, Old Tuberculin test was positive, the sputum culture was positive for *Pseudomona aeruginosa.* EKG showed sinus rhythm, left axis deviation, low Tl suggestive of old myocardial disease. Hemoglobin – 15.6 gm %, W.B.C. – 6400 with normal differential, ESR – 46 mm/hr.

He was treated with digitalis, diuretics, antibiotics and bed rest, and discharged after 1 month.

Over the next year he had a steady downhill course, with two hospital admissions for increased dyspnoea and weakness associated with fever. Clinically he showed bilateral rales, increased sputum, and chest X-rays suggesting pneumonitis (in addition to nodular fibrosis), with JVD, hepatomegaly and ankle edema as before. Sputum was repeatedly negative for acid-fast bacilli, malignant cells and pathogens. Routine antibiotics were not helpful, and he only improved after starting on I.N.H., P.A.S. and streptomycin. On a third admission he was dyspneic, drowsy and cyanotic with severe JVD and edema, right pleural effusion, bilateral rales, pulse of 72 (regular), and blood pressure 130/70. Arterial oxygen saturation was 71.4% and PCO_2 was 53.7 torr. Thoracentesis obtained 800 cc's of clear straw-coloured fluid, negative for pathogens and malignant cells. Chest X-ray showed the effusion on admission, and, when discharged after a prolonged course in hospital, general nodular densities up to 2 - mm. diameter plus slight bilateral hilar enlargement plus an area of density in the right lower lung field.

The patient's final admission was on July 21, 1964. At this time he was extremely dyspnoeic, able to take only 1 or 2 steps without severe difficulty even though he had continued with his digitalis, diuretics, and low-sodium diet. He was also now suffering from orthopnea and paroxysmal nocturnal dyspnoea. Generally he felt extremely weak, and his appetite had been poor for 1 month. On examination this thin wasted man was obviously dyspneic, markedly cyanosed and had severe peripheral edema. There was no clubbing of the fingers. Liver was down 8 cms., apex rate – 80 and regular, blood pressure 110/60. Chest expansion was poor and there were bilateral coarse rales. Chest X-ray showed consolidation in the posterior portion of the right lower lobe with diffuse nodularity and some cardiac enlargement. Sputum was positive for malignant cells on August 13th – two further sputums were questionable. Other laboratory findings were non-contributory.

Despite treatment with oxygen, digitalis, diuretics and antibiotics the patient did poorly and died on September 2nd, 1964.

DIFFERENTIAL DIAGNOSIS

This gentleman presented with a 30-year history of increasing dyspnea. Initially symptoms were greatly aggravated by recurrent chest infections, responded well to antibiotics and bronchodilators, and seemed to be without any elements of cardiac failure. Conditions causing such a picture for many years are fairly few in number:

1) Chronic bronchitis and pulmonary emphysema, 2) Asthma, 3) Sarcoidosis, 4) Pneumoconiosis, 5) Diffuse interstitial fibrosis, 6) Giant bullous emphysema, 7) Pulmonary tuberculosis.

The late age of onset, lack of history of allergy and non-episodic nature of the symptoms make asthma unlikely, though the wheezing and response to bronchodilators suggest it.

Pulmonary sarcoid involvement must be severe to produce symptoms, and survival for 30 years after they start is unlikely. Similarly, the course of pulmonary

tuberculosis is usually shorter; also, sputum samples were never positive for acid-fast bacilli. The lack of other symptoms removes diffuse interstitial fibrosis, associated with such conditions are rheumatoid arthritis and sclerodema, as possibilities; the diffuse interstitial fibrosis of berylliosis and asbestosis is also unlikely as this condition usually includes severe clubbing of the fingers, perhaps due to the severe alveolar diffusion deficit it causes. There is no history of spontaneous pneumothorax to suggest giant bullous emphysema. Thus, one is left with chronic bronchitis and emphysema – entirely compatible with the history – and the pneumoconioses, of which silicosis is the commonest. Silicosis typically has a long history of increasing dyspnoea; however it is not usually complicated by recurrent infection, except tuberculosis.

Clinically the diagnosis may be made by noting the history of exposure to dust during Mr. H. H.'s mining years, by the X-ray findings. Although the dust composition is not known, silica is a major component of most rock. Thus, the most likely pneumoconiosis here is silicosis and this is confirmed by the extremely characteristic diffuse nodular fibrosis seen on X-ray.

Silicosis has three major complications, two of which are usually fatal. Many silicotics develop destructive emphysema; however many more, up to 75%, develop tuberculosis. Since acid-fast bacilli often are not apparent in sputum of silicotics until tuberculous cavitation takes place, and since Mr. H. H. only responded well to the triple-therapy on his later admissions, it is not impossible that he had silico-tuberculosis. However, the ultimate cause of death was principally heart failure, likely cor pulmonale, the third major complication of silicosis.

Clinically the patient presented with right-sided heart failure 18 months before death, the left-sided elements of failure (orthopnea, paroxymal nocturnal dyspnea, cyanosis) appearing only terminally. Chronic heart failure may be due to intrinsic heart disease, such as coronary arteriosclerosis or amyloidosis in a chronically ill person – the history of myocardial infarct, EKG changes and history of chronic illness make both of these possible here. Heart failure may also be due to hypertensive dynamics, either systemic or pulmonary – these are more likely to be the cause if the failure is principally right- or left-sided, as in this case.

To preserve the law of parsimony, one must ascribe the heart failure to cor pulmonale secondary to silicosis. It should be noted that any diffuse chronic chest disease that increases pulmonary vascular resistance by actual destruction of the vascular tree or by chronic vasoconstriction due to alveolar hypoxia may cause cor pulmonale – thus his heart failure does not support the diagnosis of silicosis specifically. Other causes of cor pulmonale need not be considered here.

CLINICAL DIAGNOSIS

Decompensated cor pulmonale secondary to silicosis.

PATHOLOGICAL EXAMINATION

(1) *Gross Findings*

Autopsy was performed on an emaciated old man weighing 125 lbs. and being 70 inches in length.

Respiratory System: Both pleural cavities were partially obliterated by dense fibrous adhesions. There was a right hydrothorax of 1000 mls. Both lungs were dark with anthracotic pigment and were considerably heavier than normal, the right lung weighing 725 gms. and the left 640 gms. (the normal lung weight being 300 - 400 gms.). The upper lobes on both sides showed diffuse emphysema and honeycombing of the parenchyma. In addition there were 3 thick, hard black scars, of 3 cm. and 1.5 cm. diameter in the upper right lobe and of 1.5 cm. diameter in the upper left lobe. Pulmonary arteries showed a moderate degree of arteriosclerosis. There was also a whitish-cream tumour nodule about 1.5 cm. in diameter surrounding a small bronchus in the central portion of the right lower lobe. The mediastinal lymph nodes were large, hard, gritty and a blackish-grey colour with fine emery-cloth appearance of the cut surface.

Cardiovascular System: The heart was large weighing 445 gms. (normal: 300 - 350 gms.), the increase being primarily due to a very large, dilated and hypertrophied right ventricle, the right ventricular myocardium measuring 8 mm. thick (normal: 3 - 5 mm.). The fossa ovalis was closed. There were no malformations of septae or vessels. The left ventricular myocardium was 1.2 cms. thick (normal 1.3 - 1.5 cm.) and there was an old healed infarction site in the upper portion posteriorly. The coronary ostia were normal and patent; the coronary arteries were normally positioned, and showed a moderately severe degree of arteriosclerosis, especially the left circumflex artery. There was severe arteriosclerosis of the aorta. All valves were normally formed and preserved.

Other Organs: The liver weighed 1280 gms. and looked grossly normal. The cut surface revealed numerous greyish-tan tumour nodules from 0.8 to 4 cm. in size; in addition the surface had a nutmeg appearance indicative of chronic congestion; the presence of easily expressible dark blood indicated acute congestion.

The spleen weighed 190 gms. (normal: 150 gms.) and was moderately firm in consistency. The appearance of the cut surface was suggestive of acute and chronic passive congestion.

(2) *Microscopic Findings*

Lungs: Sections of right and left lungs exhibit the presence of numerous nodular areas of fibrosis of variable size, disposed subpleurally, around smaller and large blood vessels and bronchi, with the smallest nodules in alveolar septal walls. The largest areas of fibrosis are comprised of numerous nodules which have aggregated. A definite lamination of collagenous connective tissue in concentric fashion, highly suggestive of silicosis is

evident in all these lesions. Variable amounts of anthracotic pigment is disposed within these nodules particularly toward the periphery where the pigment is present in numerous macrophages. In many places the fibrosis described above involves blood vessels, producing an endarteritic type of obstruction. In a few areas, there is actual destruction of blood-vessel walls by the fibrotic process. In the largest blood vessels, pulmonary hypertension is manifested by an atherosclerotic change of moderate degree. The lungs generally show a severe destructive emphysema with formation of fairly large centrilobular spaces. Alveolar walls show a variable amount of collagenous thickening, and everywhere there are accumulations of macrophages containing large amounts of brown-black pigment. Smaller bronchi and bronchioles are surrounded by such aggregations of macrophages and in several instances this has evoked a granulation tissue type response. In addition, several bronchioles exhibit acute and chronic inflammatory changes, but generally their epithelial linings are intact.

In a few of the larger areas of silicotic fibrosis there is central necrosis and accumulation of cholesterol crystals. Nowhere is there definite histologic evidence of active tuberculous infection.

In addition, sections taken from the right lower lobe show a carcinoma apparently arising from a smaller bronchus and infiltrating fairly widely into the adjacent lung parenchyma. The tumour, adjacent to what appears to be the bronchus of origin is quite anaplastic in appearance but more distally takes on a definite epidermoid appearance. Elsewhere, tumour cells line greatly thickened alveolar septae and in these areas the malignancy has a definite adenoid appearance.

Sections of lymph nodes from the mediastinum show large aggregations of macrophages containing anthracotic pigment. There is a mild fibrosis associated with these collections but typical lesions of silicosis are not seen.

In summary, the lungs exhibit numerous widely scattered silicotic nodules of varying size, associated with marked anthracotic pigmentation and severe destructive emphysema, mainly of the centrilobular type. An associated fairly widespread arterial change is also noted in conjunction with the above, and this is chiefly in the nature of an endarteritis obliterans. The carcinoma found in the right lower lobe appears to be unrelated to the silicotic disease.

Other Organs: Liver sections showed infiltrating secondary carcinoma of a type duplicating the bronchogenic carcinoma. They also confirmed acute and passive congestion, as did sections of the spleen.

The bone marrow showed moderate erythroid hyperplasia, presumably in response to the patient's chronic hypoxic state.

(3) *Pathological Diagnosis*

(a) Cor Pulmonale – secondary to silicotic pulmonary fibrosis and destructive emphysema.
(b) Bronchogenic Carcinoma – not a direct cause of death.

DISCUSSION – PNEUMOCONIOSIS

In considering lung disease caused by exposure to dust two major factors are involved: firstly, dust in general may non-specifically predispose the lungs to various conditions and, secondly, a specific dust may cause a specific reaction, or pneumoconiosis, in the parenchymal tissue.

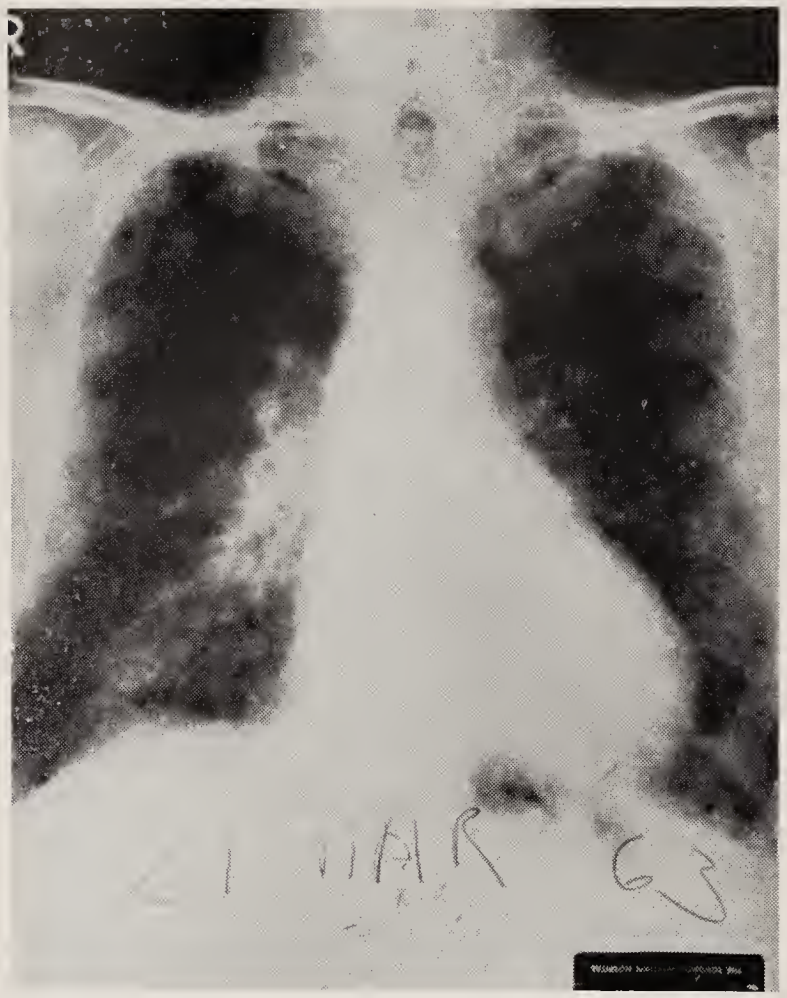
Non-specific effects are due to irritation and the fact that the dust must be cleared from the lungs, either in mucus moved by cilia up the bronchial tree, or via lymphatics as free particles, or in macrophages. Prolonged irritation may first cause greatly increased mucus production, then goblet-cell metaplasia, loss of cilia and eventual destruction of respiratory epithelium, thus predisposing to partial obstruction and small foci of infection. Similarly aggregations of dust-laden macrophages may partially obstruct bronchioles causing stenosis, bronchiolectasis (bronchiolitis deformans pneumoconiotica), and the subsequent multiple minor foci of bronchopneumonia so often found in pneumoconiotic lungs. These factors may account acutely for considerable respiratory symptomatology without much fibrous change (or radiographic change), and chronically for developing emphysema. This is the common picture with coal-dust pneumoconiosis without silicotic fibrosis.

The specific reactions to different dusts are various and generally not well explained. Cotton dusts gives a predominant picture of bronchitis & emphysema (Byssinosis); coal dust appears to cause no specific change; beryllium, after only short exposure, may cause acute inflammation or a chronic cellular granulomatous reaction indistinguishable from that of sarcoid (Berylliosis); asbestos fibres, after long exposure, cause a diffuse interstitial fibrosis (Asbestosis) and predispose to lung cancer (up to 10% of cases). Several other pneumoconioses are well known, but the commonest of all is Silicosis, which will now be discussed in more detail.

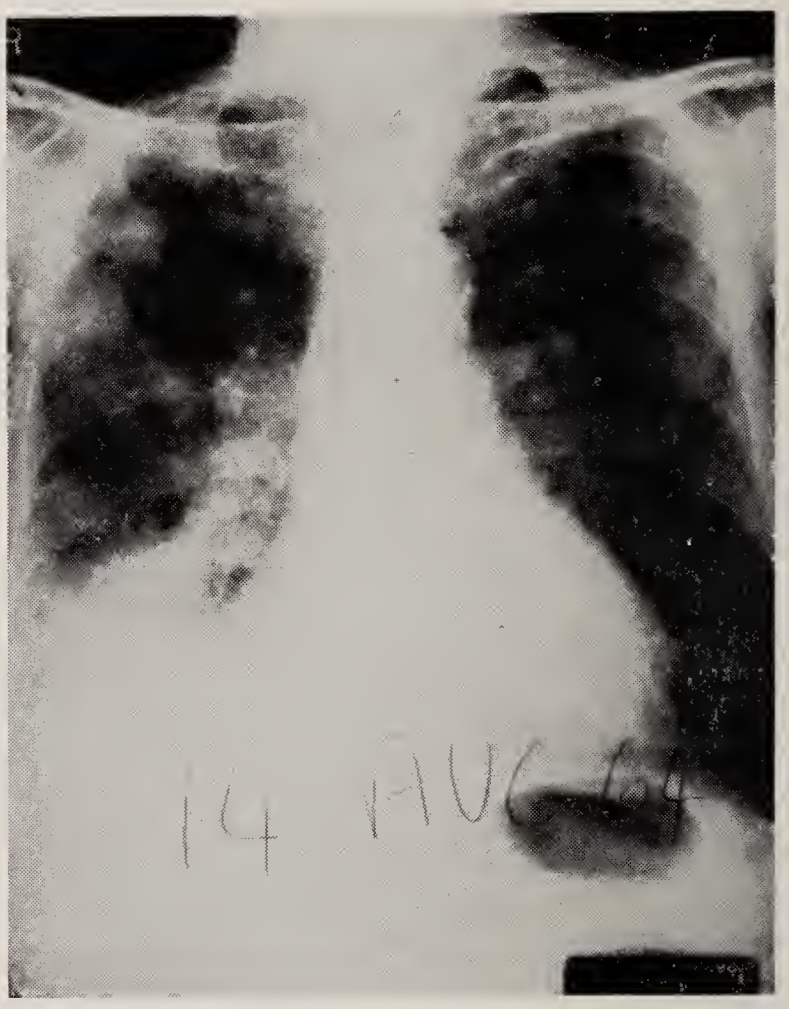
Silica dust causes a typical nodular fibrosis as described in the pathological discussion of this cases. Factors in extent and type of this fibrosis are as follows.

(1) *Particle type and size:* Only free forms of silica are fibrogenic. They are all 100% SiO_2 but have various molecular arrangements, and may be crystalline such as quartz and cristobalite, or amorphous such as vitreous silica and the silica of diatomite. Their fibrogenicities vary greatly for unknown reasons. Other minerals such as mica, talc, kaolin, contain a high percentage of SiO_2 but none of these silicates, except asbestos, is fibrogenic. Asbestosis is likely due to another mechanism.

Only particles less than 10μ long reach the alveoli, but a wide range of sizes less than 10μ are fibrogenic



March 21, 1963. P-A view of chest showing widespread nodular densities up to 2-3mm. in diameter, increased linear markings from periphery to hilar areas, slight bilateral enlargement, and an area of density in the right lower lung field.



August 14, 1964. P-A view of chest showing increased area of consolidation in the right lower lobe and cardiomegaly in addition to the nodularity seen on previous films.

(from quartz at 1μ - 3μ to the 0.1μ particles from diatomite), so that, in general, fibrogenicity is not related to size.

(2) *Mixture with other dusts:* Other dusts may have their own effects, but may also moderate the effects of silica. Aluminum dust appears to coat silica making it non-fibrogenic, the basis for aluminum dust prophylaxis against silicosis. Gypsum dust seems to reduce the effect of quartz particles by coating them such that they are too large to significantly reach the alveoli.

(3) *Concentration of dust and duration of exposure:* Unless the dust concentration is very high and exposure intense, silicosis will take 10 - 15 years to develop. In any case it takes at least several years of exposure to develop, in contrast to some other pneumoconioses, such as berylliosis. Continued exposure to silica is not required for rapid progression of the silicotic process once started.

(4) *Health and constitution of individual:* There are many other factors here which cannot be measured. One major influence on any chronic chest disease is smoking and this may greatly effect the symptomatology of silicosis if not the pathological changes.

The above factors considered, the remarkable biological activity of silica, so chemically inert, has to be explained. Early theories included mechanical irritation by the sharp hard particles, and irritation by piezoelectric charges (charges developed by mechanical stressing of the crystals) – these theories have been largely discarded.

In 1947, the silica solubility theory was proposed – solution of the silica particles producing silicic acid which has a cytotoxic effect, stimulating collagen formation. However, later work showed that fibrogenicity and solubility of various silicas did not correlate. Also, only polysilicic acids were found to have a cytological effect, whereas only monosilicic acid is produced on dissolution of quartz.

Current interest centres on adsorption, surface structure and immunological effects. Some work has suggested that silica absorbs and denatures tissue proteins causing cell death; subsequent autolysis releases the particles for recycling of the process, setting up a chronic irritation and exciting a fibrous response. A variant of this theory proposes that rather than denature tissue proteins, silica within macrophages causes regressive cellular changes (by virtue of its surface activity) which lead to production of cellular lipoproteins. On death and lysis of the macrophages, the liberated liproproteins, being antigenic, excite an antibody response. The hyaline centres of silicotic nodules are known to contain considerable amounts of Beta and gamma globulins de-

rived from plasma, thus supporting the latter theory. Despite the theories considered above, the exact nature of the pathogenesis of silicotic fibrosis is still not positively known.

It was previously noted that silicosis will progress without continued exposure to silica. The large areas of fibrosis seen in late silicosis are the result of this progression and are conglomerations of extended silicotic nodules. Extension presumes that silica particles have moved through the lung parenchyma – this most likely occurs during infections, the sequestered particles being released and moving with edema fluid toward unaffected alveolar spaces. Thus new foci of fibrosis will arise, especially adjacent to pre-existing nodules where the fibrous stiffness reduces respiratory excursion of adjacent alveoli and hence their ability to clear themselves of dust-filled macrophages which proliferate, desquamate, and eventually lyse in the alveoli, thus initiating the fibrotic process.

In conclusion, some possible mechanisms of pathogenesis of silicotic fibrosis have been reviewed. However, as well illustrated by the case presented, gross fibrosis is not the only cause of respiratory difficulty and developing cor pulmonale in silicosis. Widespread minimal fibrosis occluding small pulmonary arteries, and moderately severe emphysema were significant factors in this man's deterioration. While these were likely due to a silicotic fibrosis, one cannot exclude non-specific dust reaction, chronic infection, and smoking as major contributors which do not show up on radiographic examination.

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ACKNOWLEDGEMENTS

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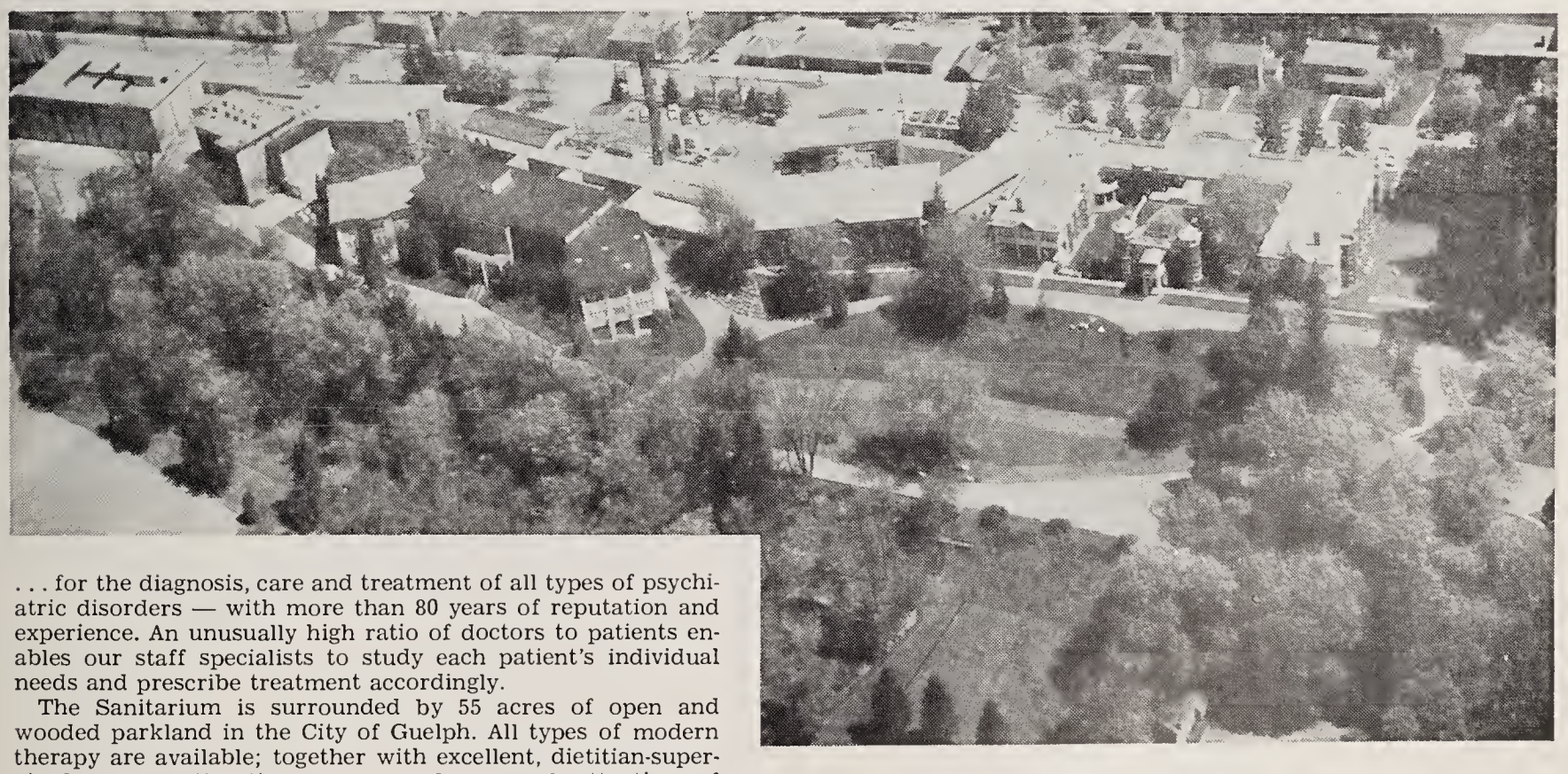

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A large and well-planned building, embracing the latest in modern Occupational therapy facilities, has recently been added. Entertainment, Motion Pictures, Bowling Alleys, Badminton and other Games, are among the Recreational activities being carried on.

Benefits, under Ontario Hospital Insurance and other group insurance plans are available to patients — depending on the nature of the illness and other factors.

For information write, or telephone Guelph 824-1010.

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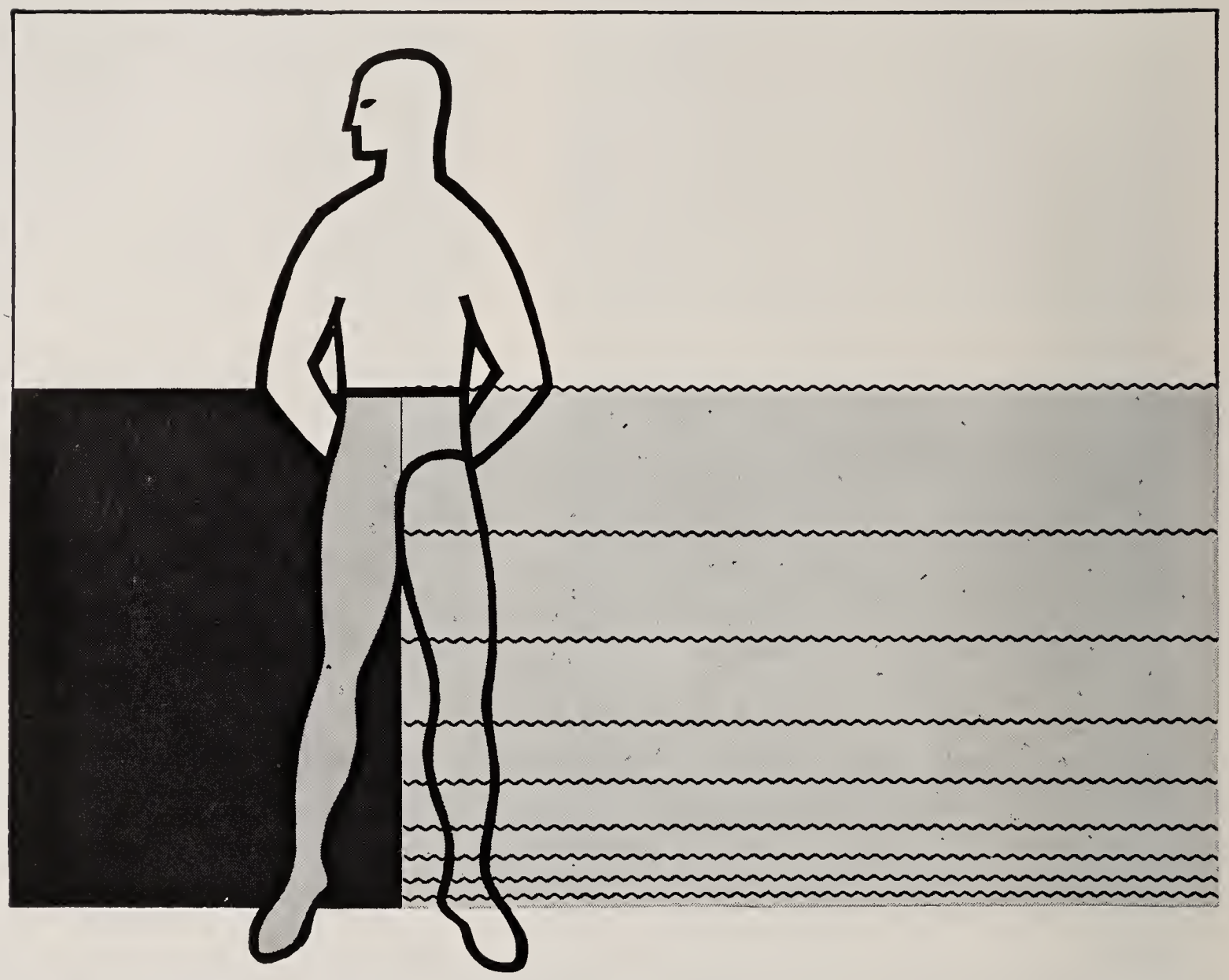

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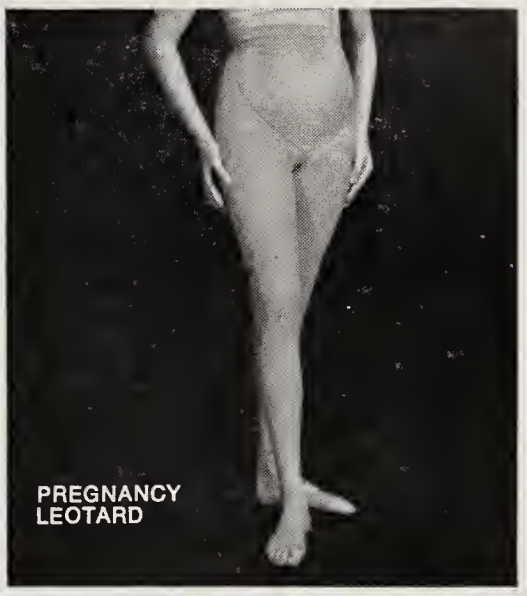
The JOBST Venous Pressure Gradient support is carefully engineered to duplicate these natural hydrostatic pressures. Each JOBST support is individually measured and custom fitted to your prescription to provide the best therapy for your patients with venous insufficiencies of the extremities.

Available for purchase or rental—JOBST Intermittent Compression Unit for treatment to reduce edema of the extremities.

Now-there's a Jobst Service Center in your area



We have opened this Service Center to make it easier for your patients to be measured and fitted with the JOBST supports.

Our Service Center is staffed with experts who will measure and fit your patients only on your prescription. (There is no charge for this service.) The exacting measurements will be engineered to provide the correct counterpressures required for each patient and his specific condition. Each support is completely guaranteed for results, material and workmanship.

To maintain the personal care and manufacturing precision that become a part of each JOBST support, we supply the physician and his patient on a direct basis. This JOBST standard of excellence and service is provided through our Service Centers, now located in major cities.

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Vitamin A..... 2500 I.U.	Vitamin B_1..... 1 mg.
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Dosage: Infants and children under 6 years of age, 5 drops daily.

Bottles of 8, 15, 30 and 50 cc., each with calibrated dropper for waste-free dosage.

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